HansaBioMed Life Sciences

Pioneering the Extracellular Vesicle field

Catalog of products and services

Excellence in life Science

Denmark

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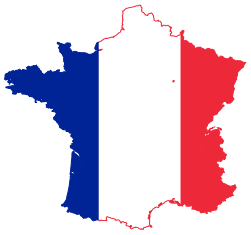
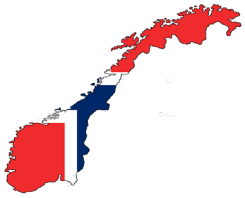


Switzerland

Norway



Distribution of HansaBioMed Life Sciences products

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HansaBioMed Life Sciences

HansaBioMed Life Sciences, founded in 2007, was the first company entirely focused on the research in the field of exosomes, extracellular vesicles (EVs) and nanoparticles. HansaBioMed, today, collaborates with various international research groups, both Academic and Industrial, in projects aimed to implement the knowledge on the full potential of the extracellular vesicles.

With our expertise, gathered in over 10 years of activity, we provide our customers with high quality of services and the widest portfolio of products dedicated to the research in exosomes and extracellular vesicles.

Our facilities are located in Tallinn (Estonia), in the Tallinn Technology park Tehnopol.

Since 2019, HansaBioMed Life Sciences is part of the Exosomics group. Exosomics S.p.A is a company founded in 2011 by the same founders of HansaBioMed and focused on the usage of the EV potential for diagnostic.

exosomics

Exosomics Group, Investors and founders

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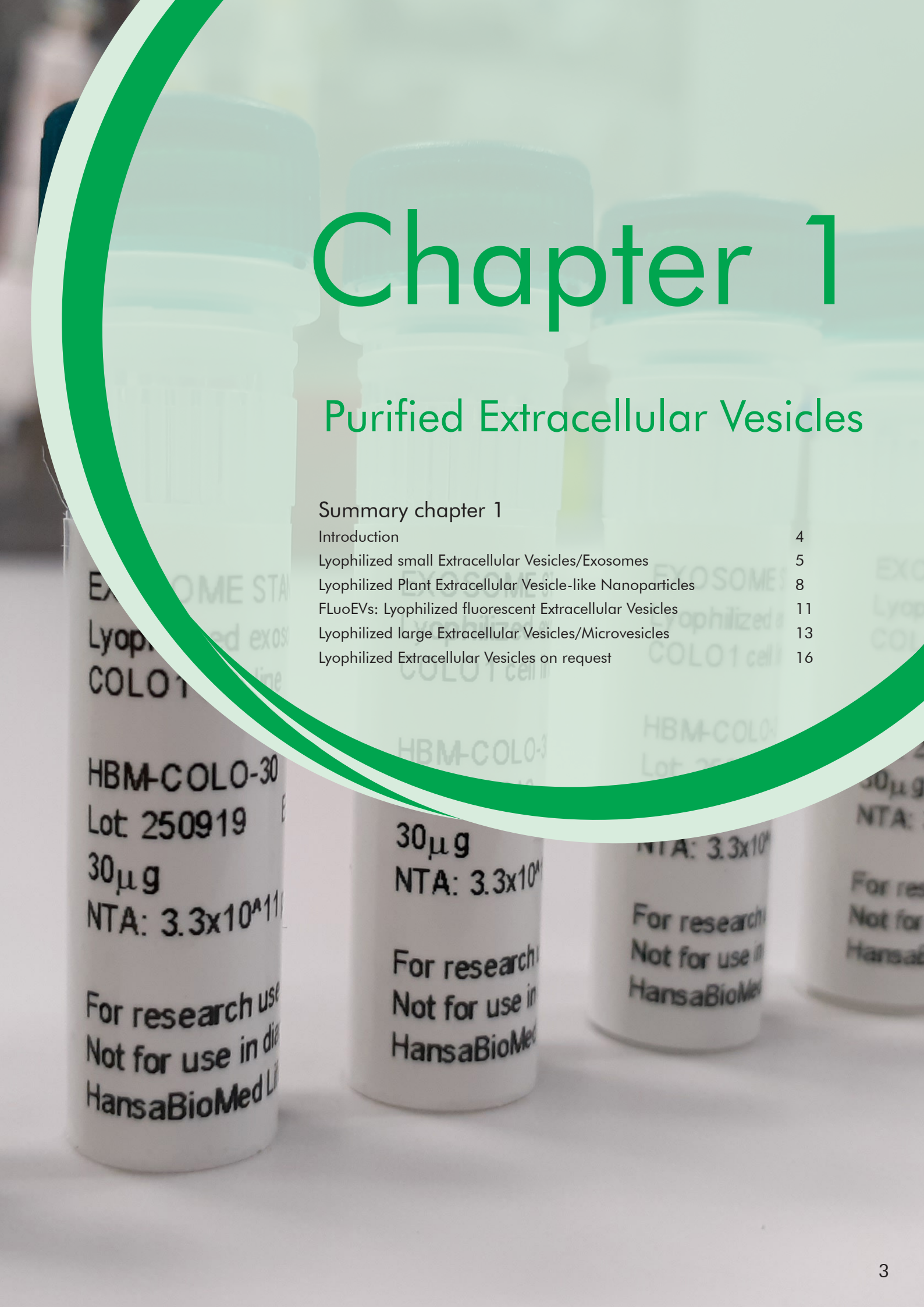
Summary

Chapter 1

Purified Extracellular Vesicles

Summary chapter 1

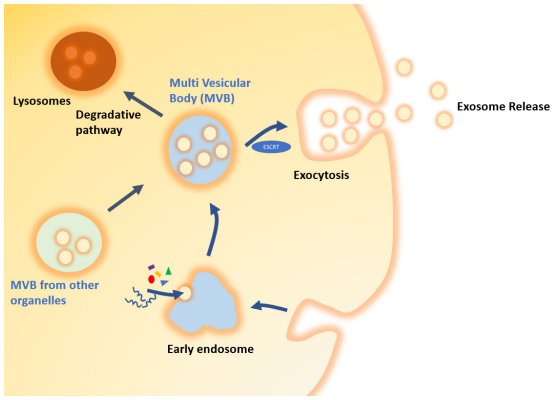


Purified Extracellular Vesicle

Introduction

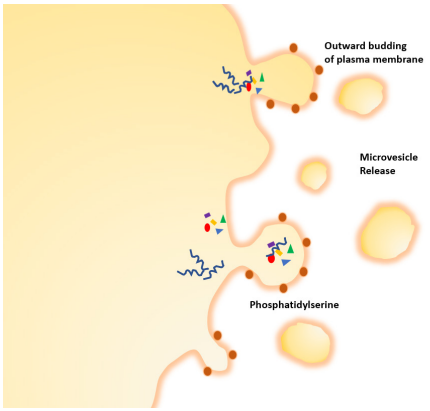
Extracellular vesicles (EVs) are cell-derived nanoparticles, differing in their cellular origin, biogenesis mechanism, size and molecular content. Even though the nomenclature of EVs is still lacking consensus, the most prominent types of EVs are Exosomes, shedding Microvesicles (MVs) and Apoptotic Bodies. Recently, EVs have started to emerge as an important mean of intercellular communication and attract more attention as diagnostic, prognostic and therapeutic tool, due to their biomarker potential, virtue to represent physiological status of parent cell and ability to modulate functions of recipient cells.

Exosomes and Microvesicles, small and large EVs: a note on the nomenclature



Exosomes are EVs with a diameter ranging from 40-120 nm that are secreted by most eukaryotic and prokaryotic cells. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions. Both quantity and molecular composition of released exosomes depend on the physiological state of the parental cells.

Microvesicles, also Ectosomes, are formed by the outward budding of the plasma membrane. Their dimensions are between 100 and 1000 nm. The release is promoted by the translocation of residues of phosphatidylserine on the external layer of the plasma membrane. During the formation process, MVs accumulate proteins and genetic material of the parental cells.



NOMENCLATURE OF PURIFIED EXTRACELLULAR VESICLES

The ISEV (International Society of Extracellular Vesicles) community defined the minimal information for studies of extracellular vesicles (MISEV guidelines*), calling Small EVs the vesicles with diameter between 30 - 120 nm and Large EVs the largest vesicles (> 120 nm). The current nomenclature was adopted because, although they differ in biogenesis, part of the Exosomes and Microvesicles secreted by the cells overlap in size and all the current available technologies do not allow to efficiently separate the two EV populations. Neverteless a wide part of the scientific community is still using the old nomenclature. In order to be conform with the MISEV guidelines, in this catalog we will use the following terms:

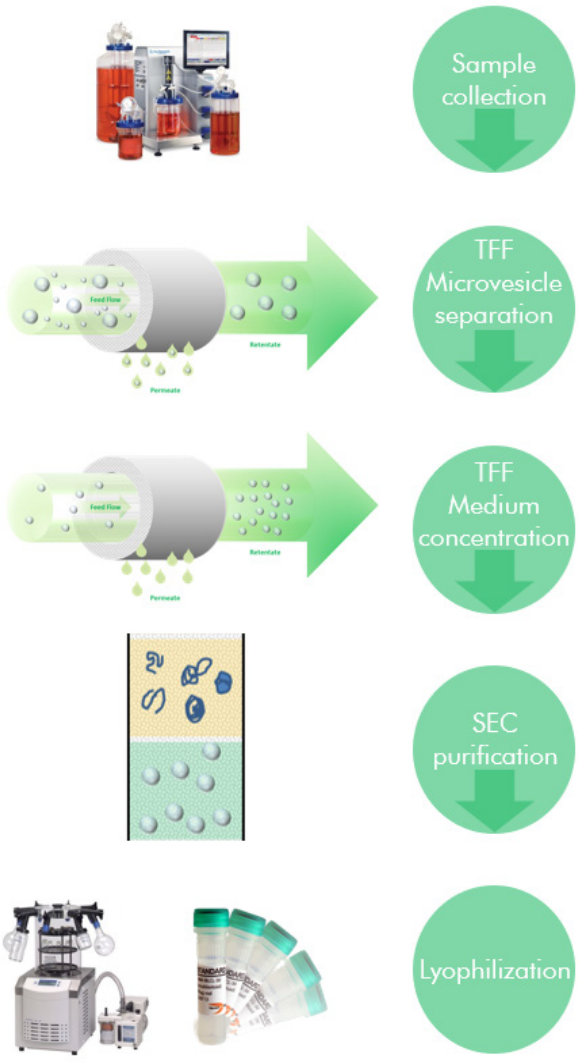
SMALL EVs (s-EVs)/EXOSOMES: vesicles with diameter comprised between 40 and 120 nm, mostly including Exosomes but also small microvesicles originated by the cell membrane.

LARGE EVs (l-EVs)/MICROVESICLES: vesicles larger than 150 nm diameter, mostly microvesicles originated by the cell membrane.

* Witwer, K. W., Soekmadji, C., Hill, A. F., Wauben, M. H., Buzás, E. I., Di Vizio, D., ... & Lötvall, J. (2017). Updating the MISEV minimal requirements for extracellular vesicle studies: building bridges to reproducibility.

Lyophilized small Extracellular Vesicles (s-EVs)/Exosomes

Purified s-EVs are obtained from a variety of biological sources: cell culture supernatant, human plasma, serum, urine. s-Evs are purified following a combination of tangential flow filtration (TFF) and size exclusion chromatography (SEC). Subsequently, isolated vesicles are quantified and validated for overall protein content, size distribution, concentration and EV specific marker expression.



Lyophilization is the ideal technique for long-term storage of EVs at 4° C. Stability of the physical properties, functionality and EV-specific marker expression of s-EVs is verified. s-EVs can be easily reconstituted by adding the appropriate volume of deionized water (MilliQ).

Applications

- Positive control for marker assessment.
- Control (spike-in) for EV quantification.
- OMICS analysis.
- Standardized positive controls for immunocapture performance evaluation.
- Flow cytometry.
- Electron microscopy.

Characteristics

- High purity.
- Size distribution: 50 - 120 nm.
- Long term stabiltiy at 4-8° C.
- Purified from biofluids collected from certified donor pools.
- Purified using combination of TFF and SEC.

Advantages

- Easy to reconstitute.
- Easy to ship and store (+4°C).
- Long term storage stability (36 months).
- Marker characterized
- Available from a large cell line bank (list of available cell line at page 16).

Purified Extracellular Vesicle

Lyophilized Small EVs/Exosomes from Human Biofluids

Cat Code	Contents	Package size (#)	
Lyophilized Small EVs/Exosomes from Human Plasma of healthy donors			
HBM-PEP100/#	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-PEP30/#	30 µg (>1x10^8 particles)	2 vials	5 vials
Lyophilized Small EVs/Exosomes from Human Serum of healthy donors			
HBM-PES100/#	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-PES30/#	30 µg (>1x10^8 particles)	2 vials	5 vials
Lyophilized Small EVs/Exosome from Human Urine of healthy donors			
HBM-PEU100/#	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-PEP30/#	30 µg (>1x10^8 particles)	2 vials	5 vials
Source of human biofluids			
All the HBM-LS Extracellular Vesicles are produced by human biofluids of certified healthy donors with informed consent.			
Lyophilized Extracellular Vesicles from Human Biofluids, Bulk production			
Small EVs/Exosomes can be produced in large bulks, on customer request. For information write to: info@hansabiomed.eu			

Lyophilized Small EVs/Exosomes from Cell Conditioned Media

HBM-LS provides Small EVs/Exosome Standards from 14 different cell line in stock, listed below, and, upon request, purified Exosome Standards from over 200 cell lines (see page 16).

Cat Code	Cell Line	Description	Particle content	Package size (#)	
Lyophilized Small EVs/Exosomes from Human MSC					
HBM-MSC-100/#	Primary cell	human adipose tissue	100 µg (>1x10^10 particles)	2 vials	5 vials
Lyophilized Small EVs/Exosomes from HEK293 cell line					
HBM-HEK-100/#	HEK293	Human embryonic kidney	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-HEK-30/#	HEK293		30 µg (>1x10^8 particles)	2 vials	5 vials
Lyophilized Small EVs/Exosome from Human COLORECTAL cancer cells					
HBM-COLO-100/#	COLO1	Human Colorectal adenocarcinoma	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-COLO-30/#	COLO1	Human Colorectal adenocarcinoma	30 µg (>1x10^8 particles)	2 vials	5 vials
HBM-HCT-100/#	HCT116	Human Colorectal adenocarcinoma	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-HCT-100/#	HCT116	Human Colorectal adenocarcinoma	30 µg (>1x10^8 particles)	2 vials	5 vials
HBM-HT29-30/#	HT29	Human Colorectal adenocarcinoma	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-HT29-100/#	HT29	Human Colorectal adenocarcinoma	30 µg (>1x10^8 particles)	2 vials	5 vials
Lyophilized Small EVs/Exosome from Human PROSTATE cancer cells					
HBM-PC3-100/#	PC3	Human Prostate adenocarcinoma	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-PC3-30/#	PC3	Human Prostate adenocarcinoma	30 µg (>1x10^8 particles)	2 vials	5 vials
HBM-LnCAP-100/#	LnCAP	Human Prostate adenocarcinoma	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-LnCAP-30/#	LnCAP	Human Prostate adenocarcinoma	30 µg (>1x10^8 particles)	2 vials	5 vials

Cat Code	Cell Line	Description	Particle content	Package size (#)	
Lyophilized Small EVs/Exosome from Human LUNG cancer cells					
HBM-A549-100/#	A549	Lung alveolar adenocarcinoma	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-A549-30/#	A549	Lung alveolar adenocarcinoma	30 µg (>1x10^8 particles)	2 vials	5 vials
HBM-NCI-100/#	NCI-H1975	Lung adenocarcinoma non-small cells	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-NCI-30/#	NCI-H1975	Lung adenocarcinoma non-small cells	30 µg (>1x10^8 particles)	2 vials	5 vials
Lyophilized Small EVs/Exosome from human GLIOBLASTOMA and NEUROBLASTOMA cells					
HBM-SK-100/#	SK-BR-3	Neuroblastoma, bone marrow metastasis	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-SK-30/#	SK-BR-3	Neuroblastoma, bone marrow metastasis	30 µg (>1x10^8 particles)	2 vials	5 vials
HBM-U87-100	U87 MG	Glioblastoma-astrocytoma	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-U87-30	U87 MG	Glioblastoma-astrocytoma	30 µg (>1x10^8 particles)	2 vials	5 vials
Lyophilized Small EVs/Exosome from human chronic and acute LEUKEMIA					
HBM-K562-100/#	K562	Leukemia, chronic myelogenous	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-K562-30/#	K562	Leukemia, chronic myelogenous	30 µg (>1x10^8 particles)	2 vials	5 vials
Lyophilized Small EVs/Exosome from MELANOMA cancer cells					
HBM-MM1-100/#	MM1	Human melanoma	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-MM1-3-/#	MM1	Human melanoma	30 µg (>1x10^8 particles)	2 vials	5 vials
HBM-B16-100/#	B16F10	Mouse melanoma	100 µg (>1x10^10 particles)	2 vials	5 vials
HBM-B16-30/#	B16F10	mouse melanoma	30 µg (>1x10^8 particles)	2 vials	5 vials
Cell line bank information					
Cell line source: All HBM-LS Extracellular Veiscles are produced using Cell Lines from the Cell Bank of the Interlab Cell Line Collection of the IRCCS AUO S.Martino IST, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy. To order Cell Lines refer directly to: www.iclc.it/indexpi.html.					
Lyophilized Extracellular Vesicles storage					
Lyophilized EVs can be stored at 4°C for up to 36 months. After reconstitution EVs can be stored at -20°C for up to one month or at -80°C for up to six months. Avoid freeze and thaw cycles.					
Lyophilized Extracellular Vesicles from Cell Conditioned Media, Bulk production					
Small EVs/Exosomes can be produced in large bulks, on customer request. For information write to: info@hansabiomed.eu					

Purified Extracellular Vesicle

Green-Fluorescent labeled Small-EVs/Exosomes

Upon request all the listed EVs in catalog can be labeled with green fluorescent probes. For information about available probes and EV quantity contact us at info@hansabiomed.eu or visit our website (www.hansabiomed.eu).

Lyophilized Extracellular Vesicles like Nanoparticles from Plants (ELNs)

The presence of EV-like nanoparticles (ELNs) in plants was suggested around the late 1960s. However, only in the last decade began a growing interest on ELNs, in particular from food and cosmetic industry. HansaBioMed Life Sciences provides purified ELNs from different plant extracts, ELNs are purified by tangential flow filtration and characterized by particle size distribution and concentration, expression of TET8 marker (corresponding of mammalian CD63). Certificate of analysis reports the origin of the material.

Cat Code	Origin	Particle content	Package size (#)
Lyophilized Plant derived Extracellular Vesicles like Nanoparticles (ELNs)			
HBM-GIN-100	Ginger root	100 μg (>1x10^10 particles)	1 vial
HBM-POT-100	Potato	100 μg (>1x10^10 particles)	1 vial
HBM-ONI-100	Onion	100 μg (>1x10^10 particles)	1 vial
HBM-GAR-100	Garlic	100 μg (>1x10^10 particles)	1 vial
HBM-SEA-100	Seaberries	100 μg (>1x10^10 particles)	1 vial
Custom production			
Plant EVs can be produced on customer request from different plants, roots, stems, leaves. For information write to info@hansabiomed.eu			

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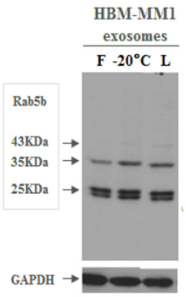
Purified Extracellular Vesicle

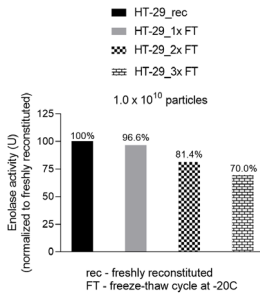
Lyophilization is the ideal method for preserving Extracellular Vesicles stability

A- MARKER EXPRESSION



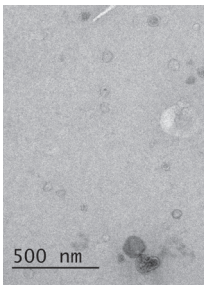
1. Fresh (F), frozen (-20ºC) and lyophilized s-EVs/

B- FUNCTIONAL PROPERTIES



2. Stability of enolase activity in lyophilized HT29 EVs (HBM-HT29).

C- MORPHOLOGY

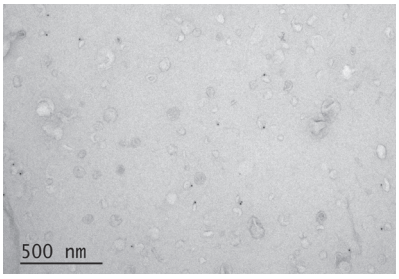


3. TEM image of lyophilized HCT116 EVS (HBM-HCT).

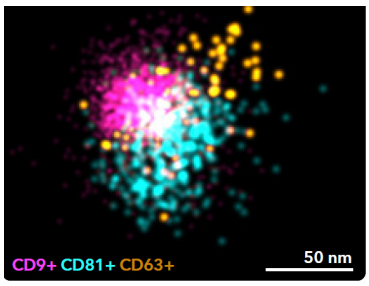
Lyophilization does not substantially affect EV particle size distribution or biomarker expression compared to other storage methods (Fig 1, 2, 3). Exosomes stored for over 3 months at -20° C or over 1 year at -80° C showed a complete different size distribution profile, probably due to EV aggregation.

Applications of lyophilized Extracellular Vesicles

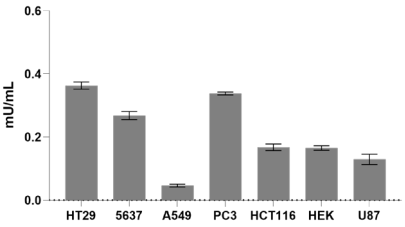
Electron Microscopy (EM) and Immuno Electron Microscopy (IEM)



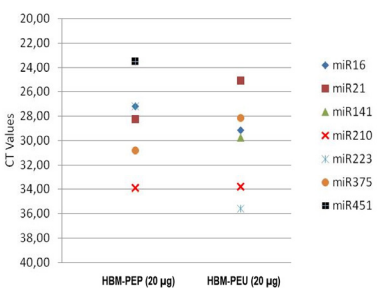
EV phenotyping by super resolution fluorescence microscopy



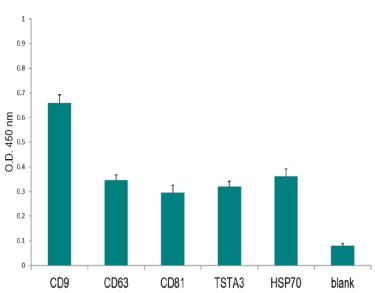
Activity assays: Acetylcholinesterase activity in lyophilized EVs



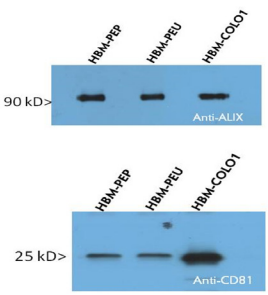
Profiling of EV associated RNAs



EV phenotyping by ELISA

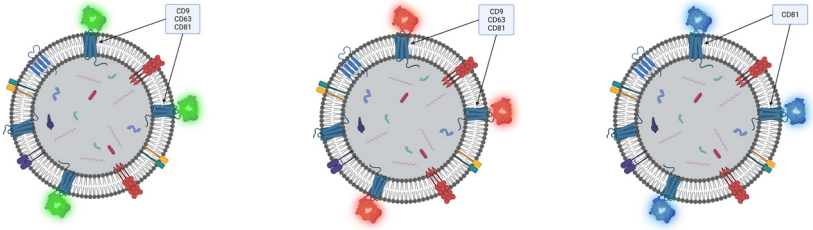


EV marker analysis by WB



FLuoEVs: Purified EVs expressing fluorescent proteins

FLuoEVs are stably-fluorescent EVs expressing the flourescent protein EGFP (green), BFP (blue) or mCherry (Red) as fusion protein with tetraspanins CD9, CD81 and CD63. FLuoEVs demonstrated high stability of the fluorophores, they can be used for *in vitro* tracking studies or as reference material for analyzers of nanoparticles or for assay calibration.



FLuoEVs are extracellular vesicles purified by combination of Tangential Flow Filtration (TFF) and Size Exclusion Chromatography (SEC) from engineered cells able to express the fluorescent proteins as fusion protein with the tetraspanin CD9, CD63 or CD81.

Cat Code	Cell origin	Fluorescent protein	Fluorescent particles/vial
FLuoEVs: Purified EVs expressing fluorescent proteins			
HBM-HEK-EFGP63	HEK293	EGFP-CD63	> 1x10^9 / 1 vial
HBM-HEK-EGFP9	HEK293	EGFP-CD9	> 1x10^9 / 1 vial
HBM-HEK-EFGP81	HEK293	EGFP-CD81	> 1x10^9 / 1 vial
Custom production			
FLuoEVs can be produced on customer request from different cell lines and different fluorescent proteins (mCHERRY/Red and BFP/Blue). For information write to info@hansabiomed.eu			

Applications

- Cell spike-in and *in vitro* tracking
- Control (spike-in) for EV quantification.
- Monitoring of EV uptake
- Standardized positive controls for EV analyzers.
- Flow cytometry.
- Fluorescence NTA.

Characteristics

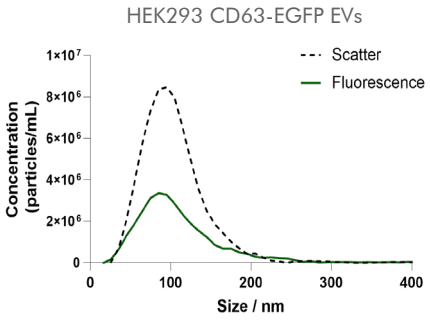
- Particle size distribution 50 - 120 nm.
- CD9, CD63, CD81 conjugated with EGFP (green) or mCHERRY (red). CD81 conjugated with BFP (blue).
- Store: 4 - 8 °C.
- Lyophilized. Reconstitution by addind deionized water.

Advantages

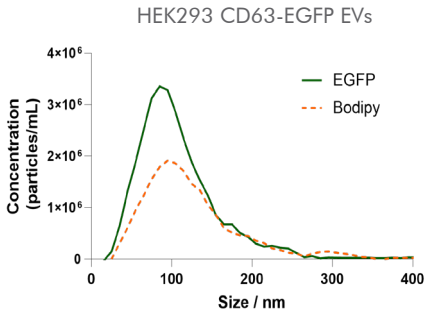
- Increrased fluorophore stability over membrane dyes.
- Easy storage and transport (4 - 8 °C).
- Custom fluorescent EVs on request.

Purified Extracellular Vesicle

Performance for FLuoEVs in fluorescence-NTA (Zetaview analyzer, Particle Metrix)

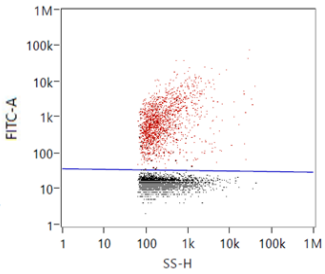


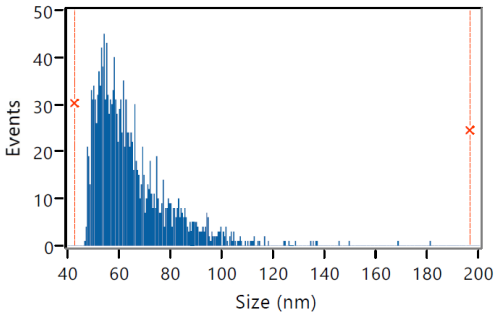
FLuoEVs HEK293-CD63-EGFP analysis in scattered vs fluorescence mode (percentage of fluorescent partilces 40 - 60 %)



Comparison of FLuoEVs HEK293-CD63-EGFP and HEK293 EVs lebeled with the lipidic dye Bodipy.

Performance for FLuoEVs in flow cytometry (NanoAnalyzer, NanoFCM)





FLuoEVs HEK293-CD63-EGFP analysis by NanoAnalyzer NanoFCM (percentage of fluorescent partilces 60 - 80 %)

Applications

- Protein marker analysis using multiple techniques.
- Extraction and analysis of MV-associated nucleic acids.
- Positive controls for NTA performance evaluation.
- Flow cytometry.
- Electron microscopy.

Characteristics

- Highly pure.
- Size distribution: 150 - 300 nm.
- Isolated by tangential flow filtration.

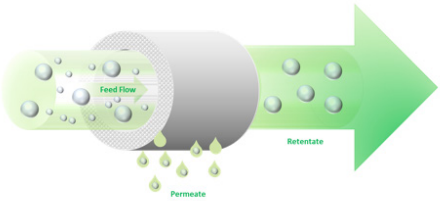
Advantages

- Easy to reconstitute.
- Easy to ship and store (+4°C).
- Long-term storage stability (36 months).
- Available on request from a large cell line bank (cell line list at page 13).

Lyophilized Large EVs/Microvesicles

Purified large EVS (l-EVs) are obtained from cell conditioned media. EVs larger than 150 nm are separated by using tangential flow filtration (TFF). Isolated vesicles are quantified and validated for overall protein content, size distribution and particle number by NTA (Nanoparticles Tracking Analysis) with Zetaview analyzer (Particle Metrix).

Sample collection



TFF Microvesicle separation

Lyophilization

Purified Extracellular Vesicle

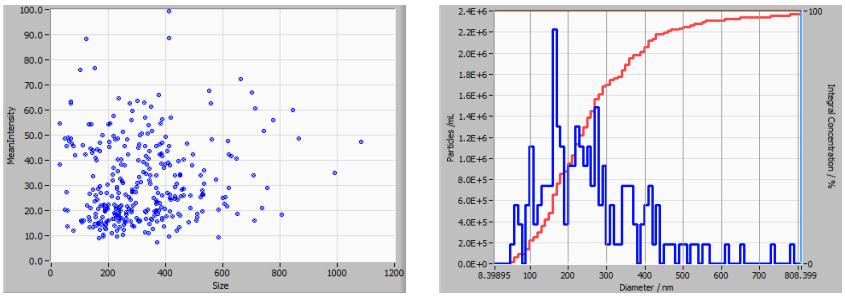
Large EVs/Microvesicles from Cell Conditioned Media

HBM-LS provides lyophilized Microvesicles from 13 different cell line in stock, listed below, and, upon request, from over 200 cell lines (see pag 12).

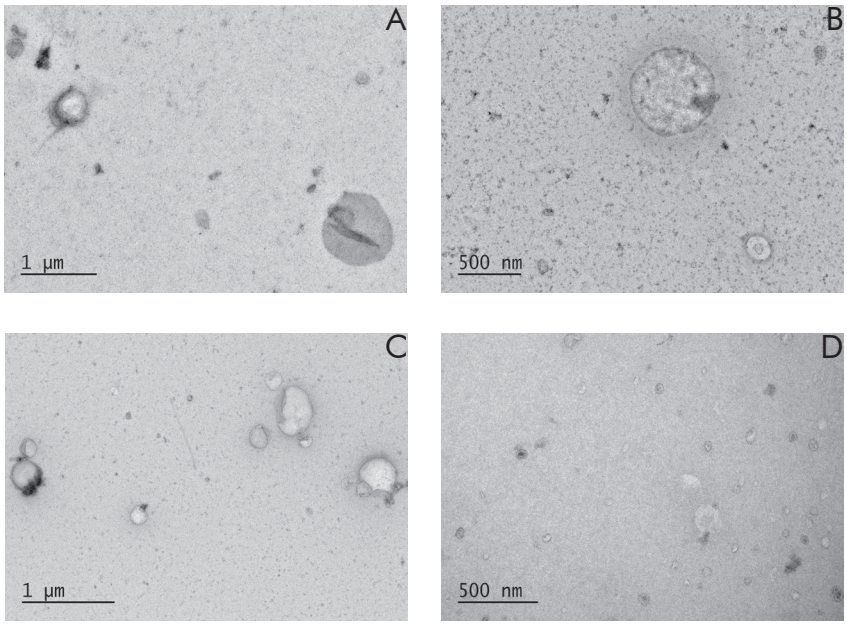
Cell Line	Code	Size
Lyophilized Large EVs/Microvesicles		
COLO1 Human colon carcinoma	HBM-mvCOLO-50	1 vials 50 µl
MM1 Human melanoma	HBM-mvMM1-50	1 vials 50 µl
U87 MG Human glioblastoma astrocytoma	HBM-mvU87-50	1 vials 50 µl
SK-N-SH Human neuroblastoma	HBM-mvSK-50	1 vials 50 µl
HCT116 Human colon carcinoma	HBM-mvHCT-50	1 vials 50 µl
PC3 Human prostate adenocarcinoma grade IV	HBM-mvPC3-50	1 vials 50 µl
A549 Human lung carcinoma	HBM-mvA549-50	1 vials 50 µl
K562 Human pleural effusion, chronic leukemia	HBM-mvK562-50	1 vials 50 µl
HEK293 Human embryonic kidney	HBM-mvHEK293-50	1 vials 50 µl
B16F10 Mouse melanoma	HBM-mvB16-50	1 vials 50 µl
Human Adipose Tissue MSC	HBM-mvMSC-50	1 vials 50 µl
Lyophilized Extracellular Vesicles storage		
Lyophilized large EVs can be stored at 4°C for up to 36 months. After reconstitution EVs can be stored at -20°C for up to one month or at -80°C for up to six months. Avoid freeze and thaw cycles.		

Application of lyophilized large EVs/Microvesicles

Lyophilized Large EVs/Microvesicles have the same versatility of small EVs, being suitable for multiple applications and techniques. Compared to the s-EVs, l-EVs show bigger dimentions and different size distribution, as revealed by NTA analysis and electron microscopy (Fig 4 and 5).



4. Size distribution profiling and scattered plot of l-EVs, performed with the Zetaview (Particle Metrix).



5. Electron microscopy images of the Lyophilized l-EVs (A,B,C) and s-EVs (D).

Purified Extracellular Vesicle

Lyophilized Extracellular Vesicles on request

Small and large EVs from the cell lines listed below upon costomer request.

List of available Cell Lines for Extracellular Vesicle purification on request		
Cell line bank information		
Cell line source: All HBM-LS Extracellular Veiscles are produced using Cell Lines from the Cell Bank of the Interlab Cell Line Collection of the IRCCS AUO S.Martino IST, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy. To order Cell Lines refer directly to: www.iclc.it/indexpi.html.		
Ordering information		
Order by email to: orders@hansabiomed.eu		
Cell Line Name	Tissue	Tumor/Pathology Description
380	peripheral blood	leukemia, pre-B cell
1301	blood	leukemia, acute lymphoblastic, T cell
5637	bladder	carcinoma
8305C	thyroid	carcinoma, undifferentiated
A 2058	skin	melanoma, metastatic
A-172	-	glioblastoma
A-204	muscle	rhabdomyosarcoma
A2780	ovary	adenocarcinoma
A-498	kidney	adenocarcinoma
A-704	kidney	adenocarcinoma
ACHN	kidney	adenocarcinoma
ACN	-	neuroblastoma
BICR 18	larynx	squamous cell carcinoma
BT-549	breast	carcinoma, ductal
BV-173*	peripheral blood	leukemia, pre-B cell
BxPC-3	pancreas	adenocarcinoma
C33A	cervix	carcinoma
CA46	ascitic fluid	lymphoma, Burkitt
Caco-2	colon	adenocarcinoma
Caki-2	kidney	carcinoma
Calu-1	lung	carcinoma, epidermoid, grade III
CaSki	cervix	cervix carcinoma, epidermoid
CFPAC-1	pancreas	adenocarcinoma
CM-S/Tum	bone marrow	monocyte tumor
CM-S/un	bone marrow	monocyte tumor
COLO 205	colon	colorectal adenocarcinoma
COLO 320DMF	colon	adenocarcinoma
COLO 699N	lung, derived from pleural fluid	adenocarcinoma
COLO 741	colon	carcinoma, pelvic wall metastasis
COLO 800	subcutaneous nodule	melanoma
COLO 853	lymph node	melanoma
COLO 858	lymph node	melanoma
* Epstein-Barr virus ** Transformed by Epstein-Barr virus *** type C, type B viruses		

Cell Line Name	Tissue	Tumor/Pathology Description
COR-L23	lung	carcinoma, large cell
DBTRG.05MG	brain	glioblastoma
DLD-1	colon	adenocarcinoma
DMS-79	lung, pleural effusion	carcinoma, small cell
DOHH2*	-	lymphoma, follicular, B cell
DU-145	prostate	carcinoma
FTC-133	thyroid	carcinoma, follicular
FTC-238	thyroid	carcinoma, follicular
G-361	skin	melanoma
GDM-1	peripheral blood	leukemia, acute myelomonocytic
GF-D8	peripheral blood	leukemia, acute myeloid
H9	lymphocyte	lymphoma
HCT-15	colon	colorectal adenocarcinoma
HCT-8	intestine, ileocecal	ileocecal adenocarcinoma
HECV	umbilical cord	-
HEL 92.1.7	-	erythroleukemia
HeLa	cervix	carcinoma, epitheloid
HeLa S3	cervix	carcinoma, epitheloid
H-EMC-SS	-	chondrosarcoma
Hep G2	liver	hepatocellular carcinoma
HFFF2	foreskin, fetal	fibroblast, fetal
HGC-27	stomach	carcinoma, undifferentiated
HL-60	peripheral blood	leukemia, promyelocytic
HOS	bone	osteosarcoma
Hs578T	breast	carcinoma
Hs913T	derived from metastasis to lung	fibrosarcoma
HT 1197	bladder	carcinoma
HT-1080	acetabulum	fibrosarcoma
HT-29	colon	adenocarcinoma, grade II
HuP-T3	pancreas	adenocarcinoma, ascitic fluid
HuP-T4	pancreas	adenocarcinoma, ascitic fluid
Hs913T	derived from metastasis to lung	fibrosarcoma
HT 1197	bladder	carcinoma
HT-1080	acetabulum	fibrosarcoma
HT-29	colon	adenocarcinoma, grade II
HuP-T3	pancreas	adenocarcinoma, ascitic fluid
HuP-T4	pancreas	adenocarcinoma, ascitic fluid
HUVEC	endothelium	umbelical vein endothelial cells
IMR-32	-	neuroblastoma
IMR-5	-	neuroblastoma
IST-MEL1	skin	melanoma
IST-MEL2	skin	melanoma
IST-MEL3	skin	melanoma

* Epstein-Barr virus
** Transformed by Epstein-Barr virus
*** type C, type B viruses

Purified Extracellular Vesicle

Cell Line Name	Tissue	Tumor/Pathology Description
IST-MELA 16	subcutaneous metastasis	melanoma, metastatic
IST-MES1	pleural effusion	mesothelioma
IST-MES2	pleural effusion	mesothelioma
IST-SL1	lung	carcinoma, small cell
KARPAS-422*	-	lymphoma, follicular, B cell
KYSE-30	oesophagus	carcinoma,squamous cell
LB4**	lymphocyte, B	paroxysmal nocturnal hemoglobinuria
LB-B7**	lymphocyte, B	paroxysmal nocturnal hemoglobinuria
LB-F9**	lymphocyte, B	paroxysmal nocturnal hemoglobinuria
LNCap.FGC	prostate	adenocarcinoma
LoVo	colon	adenocarcinoma
LS 180	colon	colorectal adenocarcinoma
M07e	peripheral blood	leukemia, acute megakaryoblastic
MCF7***	breast	adenocarcinoma
MDA-MB-415	breast	adenocarcinoma
MDA-MB-435S	breast	carcinoma, ductal
MDA-MB-436	mammary gland	adenocarcinoma
MDA-MB-453	breast	adenocarcinoma
MDA-MB-468	breast	adenocarcinoma
MeCo 05	skin	melanoma
MEG-01	bone marrow	leukemia, megakaryoblastic
MEGR 07	metastatic cutaneous nodule	melanoma
MeMo 05	lymph node, metastasis	melanoma
MEMOR 06	subcutaneous metastasis	malignant melanoma
MES-SA	uterus	sarcoma
MEWO	-	malignant melanoma
MG-63	-	osteosarcoma
MOLT-4	peripheral blood	leukemia, T cell
MONO-MAC-6	peripheral blood	leukemia,acute monocytic
MPP 89	pleural effusion	mesothelioma
MRC-5	lung, fetal	-
MSTO-211H	-	mesothelioma
NCI-H1650	lung	adenocarcinoma, bronchioalveolar carcnoma (smoker patient)
NCI-H1975	lung	adenocarcinoma, non-small cell (non-smoker patient)
NCI-H292	lung	carcinoma, mucoepidermoid
NCI-H727	lung	carcinoma, non-small cell
NT2-D1	testis	carcinoma, embryonal pluripotent
OCI-AML2	peripheral blood	leukemia, acute myeloid
PA-1	ovary	teratocarcinoma
PF-382	pleural effusion	leukemia, T cell
PSN1	pancreas	adenocarcinoma
RAJI	-	lymphoma, Burkitt
Rj2.2.5	-	lymphoma, Burkitt

* Epstein-Barr virus
** Transformed by Epstein-Barr virus
*** type C, type B viruses

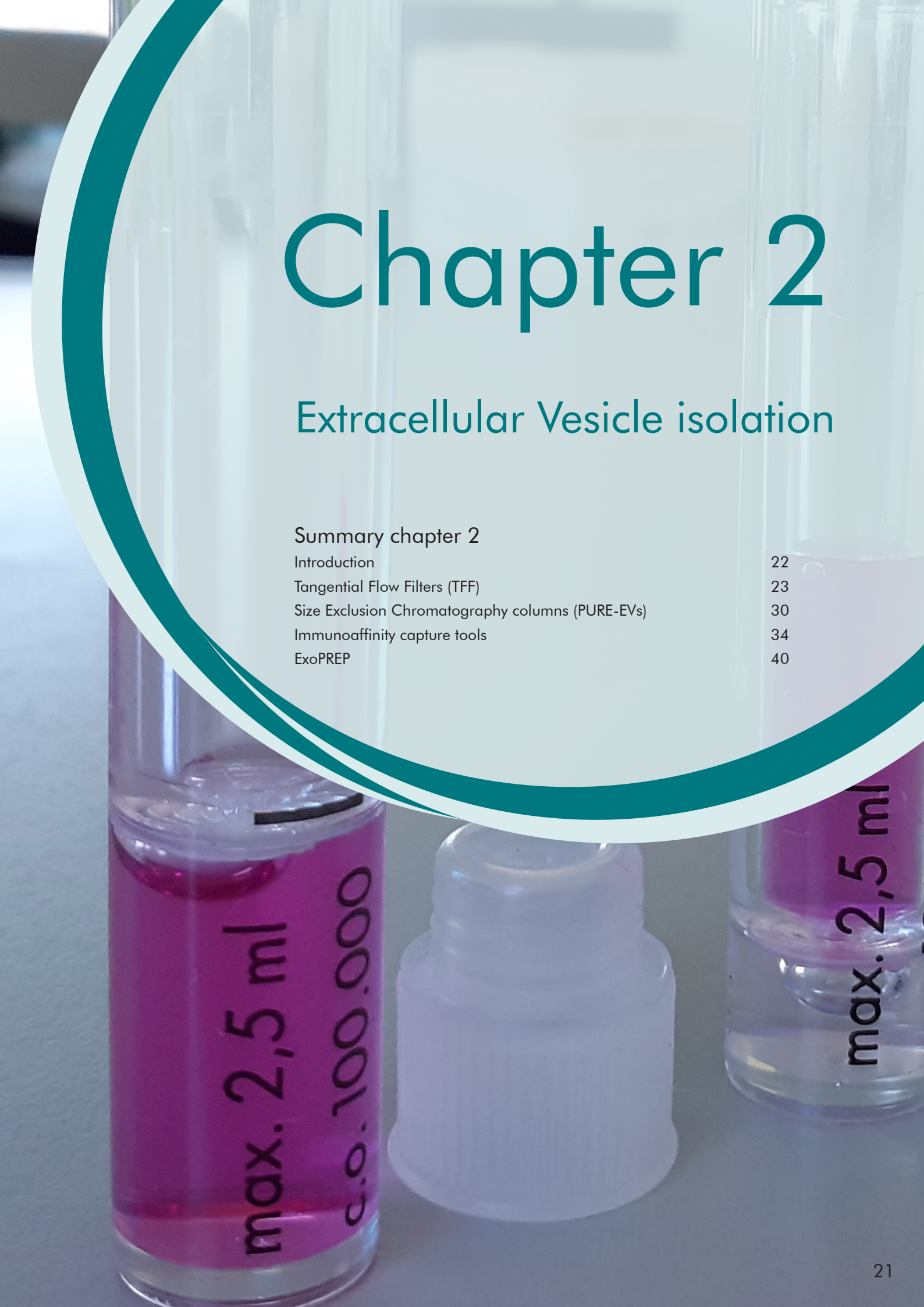
Cell Line Name	Tissue	Tumor/Pathology Description
RO82-W-1	thyroid	carcinoma, follicular
ROV-S**	bone marrow	lymphoblastoid, EBV transformed
RPMI 7932	skin	melanoma
Saos-2	bone	osteosarcoma
SH-SY5Y	bone marrow metastasis	neuroblastoma
SiHa	cervix	carcinoma, squamous cell
SK-BR-3	breast	adenocarcinoma
SK-HEP-1	liver	adenocarcinoma
SK-LU-1	lung	adenocarcinoma, grade III, poorly differentiated
SK-MEL-24	skin	melanoma
SK-MEL-28	skin	melanoma
SK-MEL-5	skin	melanoma
SK-MES-1	lung	carcinoma, squamous cell
SK-N-AS	-	neuroblastoma
SK-N-BE(2)	bone marrow	neuroblastoma
SK-N-BE(2)-C	bone marrow	neuroblastoma
SK-N-F1	bone marrow metastasis	neuroblastoma
SUP-T1	pleural effusion	lymphoma, lymphoblastic, T cell
SW1353	bone	chondrosarcoma
SW48	colon	adenocarcinoma, grade IV
SW480	colon	adenocarcinoma, grade III-IV
SW620	colon	adenocarcinoma, metastasis to lymph node
SW837	rectum	adenocarcinoma, grade IV
T47D	breast	carcinoma, ductal
T84	colon	carcinoma, metastasis to lung
TE671 Subline 2	-	rhabdomyosarcoma
TF-1	bone marrow	erythroleukemia
THP-1	peripheral blood	leukemia, acute monocytic
THP-1h	peripheral blood	leukemia, acute monocytic
THP-1l	peripheral blood	leukemia, acute monocytic
U251 MG	-	glioblastoma-astrocytoma, grade III
U87 MG	brain	glioblastoma-astrocytoma
U87/DK	brain	glioblastoma, transfected with binase (ATP binding site 721), mutated de 2-7 EGFR
U87/WT	brain	glioblastoma, transfected with EGFR
U-937	pleural effusion	lymphoma, histiocytic
VA-ES-BJ	skin	sarcoma, epitheloid
WiDr	colon	colorectal adenocarcinoma
Y79	-	retinoblastoma

* Epstein-Barr virus
** Transformed by Epstein-Barr virus
*** type C, type B viruses

Chapter 2

Extracellular Vesicle isolation

Summary chapter 2



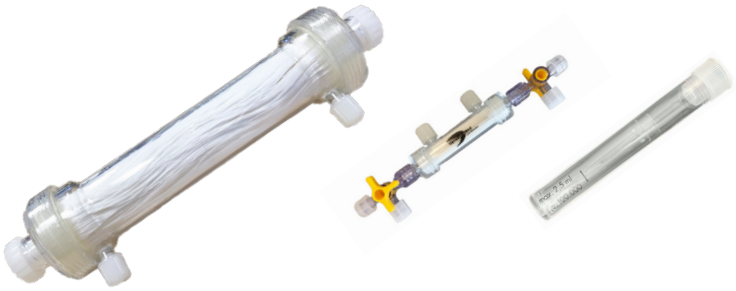
Extracellular Vesicle isolation

Introduction

Although ultracentrifugation is still widely used, it is not anymore considered the gold standard methodology for Extracellular Vesicles isolation, since it does not isolate EVs efficiently, tends to alter the vesicle shape and functionality, requires expensive equipment and is time-consuming. In more than ten years of experience, we have developed and optimized methods and tools for a fast, scalable and reproducible EV purification, for addressing the EV heterogenity and high throughput solutions for biomarker discovery.

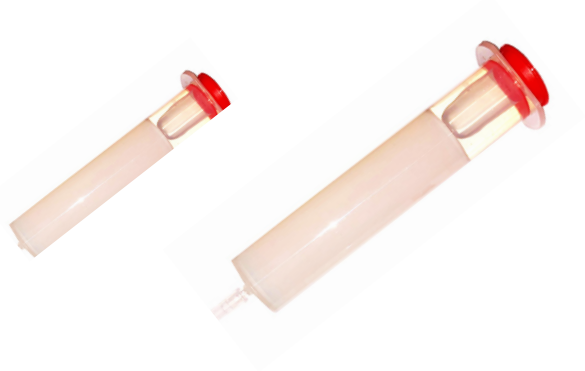
Ultrafilters

For EV size separation and medium concentration



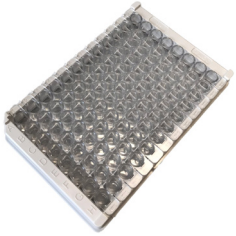
Size Exclusion Chromatography Columns

For EV purification and removal of contaminants



Immunoaffinity isolation

For biomarker screening and enrichment of EV subpopulations



Chemical isolation

For a fast and simple EV precipitation from small volume of fluids

Tangential Flow Filters for EV concentration and purification

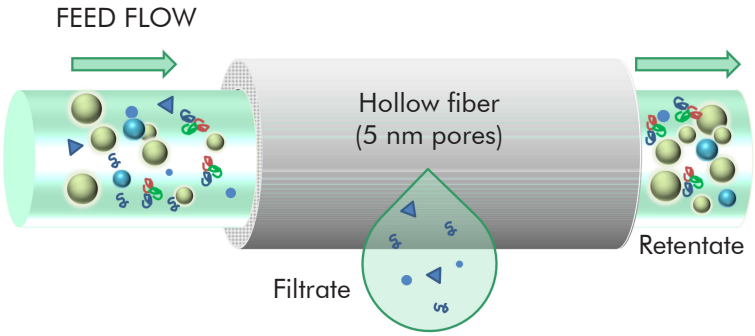
Tangential flow filtration (TFF) is a rapid and efficient method, usually used for separation and purification of biomolecules in industrial applications and can be successfully applied to isolate and separate extracellular vesicles. We provide three different typologies of filters suitable respectively for EV purification, EV concentration and Ev size-based separation.

Characteristics

- TFF-EVs, pore zise: 50 nm.
- TFF-Easy pore size: 5 nm.
- TFF-MV pore size: 200 nm.
- Suitable for manual or mechanical use.

TFF-Easy: Filter for EV concentration and dialysis

TFF-Easy is a filter cartridge in hollow fibers made of polysulfone, which allows the particle concentration and the removal of small proteins and molecules from diluted matrices (cell conditioned media, urine, etc..), prior to the EV purification.



The small dimensions of the device allow to concentrate samples from 5 ml up to bigger volumes, surmounting the limit of the TFF technique which is usable for processing big volumes of fluids.

Applications

- Concentration of diluted fluid as cell media or urine prior to EV isolation.
- Easy removal of small molecules and ions from the EV preparation.
- EV dialysis and buffer exchange.
- High efficiency of EV isolation if coupled with SEC columns.

Advantages

- Washable.
- Reusable multiple times.
- Easy to use.
- Fast concentration of EV containing matrices.

Cat. Code	Filter Volume	Quantity
TFF-Easy: EV concentration and dialysis		
HBM-TFF/1	2 ml	1 filter
HBM-TFF/5	2 ml	5 filters

References

Chetty, V. K., Ghanam, J., Anchan, S., Reinhardt, K., Brenzel, A., Gelléri, M., ... & Thakur, B. K. (2022).

Pansani, T. N. N., Phan, T. H. H., Lei, Q., Kondyurin, A., Kalionis, B., & Chrzanowski, W. (2021).

Woith, E., Guerriero, G., Hausman, J. F., Renaut, J., Leclercq, C. C., Weise, C., ... & Melzig, M. F. (2021).

Phan, T. H., Divakarla, S. K., Yeo, J. H., Lei, Q., Tharkar, P., Pansani, T. N., ... & Chrzanowski, W. (2021).

Extracellular Vesicle isolation

Applications

- Separation, concentration and recovery of large EVs (>150 nm).
- Large EV isolation from cell media, biofluids, plant extracts.
- Dialysis and desalting of large EVs.
- Suitable for large EV isolation from 5 ml of fluid.

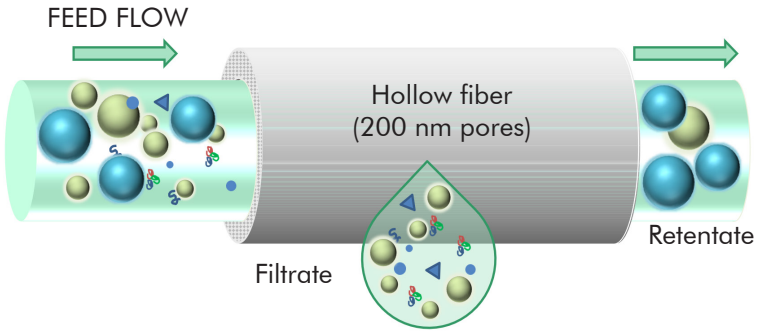
Advantages

- Washable.
- Reusable multiple times.
- Sterile
- Fast separation of EVs bigger than 150 nm.

TFF-MV: Filter for large EV separation and purification

TFF-MV is a filter able to separate large microvesicles (MVs) by size, avoiding the separation by centrifugation at 10000g, which often causes the loss of part of small EVs. TFF-MV retains vesicles larger than 150-200 nm, whereas small EVs and circulating molecules pass in the permeate. Retained MVs can be recovered with a syringe in PBS buffer, without additional purification steps.

TFF-MV is a filter cartridge made of hollow fibers with pores of 200 nm size. It can be used manually with syringes and allows the separation of large microvesicles from small EVs (< 150 nm). It works from a mimimal amount of 5 ml of fluids up to liters of fluids.

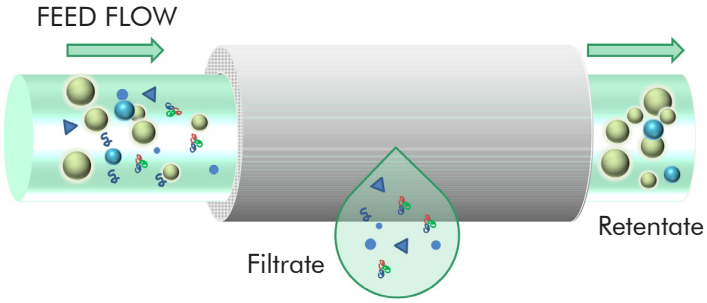


Cat. Code	Filter Volume	Quantity
TFF-MV: Separation concentration and purification of large EVs		
HBM-TFF-MV	2 ml	1 filter
HBM-TFF-MV/5	2 ml	5 filters

TFF-EVs: Fast EV purification

TFF-EVs is our next-generation filter which allows a rapid, reproducible and scalable purification of EVs, can be used on the lab bench for purifying small amount of samples (min 5 ml) or connected with a mechanical system for purifying larger volumes.

TFF-EVs is a filter cartridge made of polyethersulfonehollow fibers with pores of 50 nm size (cut off 300 kDa). The filter allows the purification of EVs and particles > 50 nm.



Avaialble in 2 sizes, the TFF-EVs Small is suitable for manual use on lab bench or under safety cabinet and allows EV purification from conditooned media or biofluids in few minutes.

The TFF-EVs Large is adapt for large scale purification and is suitable for mechanical use by peristaltic pump.

TFF-EVs Small

TFF-EVs Large

Cat. Code	Filter Volume	Quantity
TFF-EVs: Filter for EV purification		
HBM-TFF-EVs-S	2 ml	1 filter
HBM-TFF-EVs-L	30 ml	1 filter

Applications

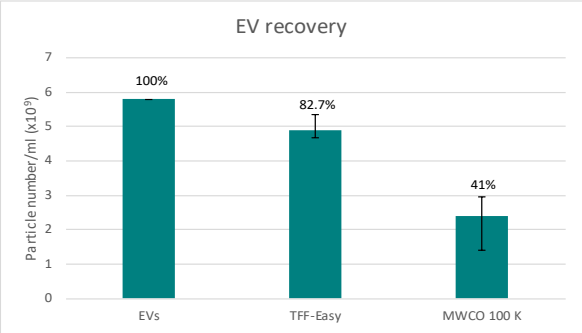
- Separation, purification and concentration of EVs from conditioned media, biofluids, plant extracts.
- Buffer exchange and removal of unbound dye.
- Depletion of FBS from bovine EVs.

Advantages

- Washable.
- Reusable multiple times.
- Sterile

Extracellular Vesicle isolation

TFF-Easy: Concentration of diluted fluids with minimal loss of EVs



1. CD81 expression in concentrated vs not concentrated CCM.

30 ug of purified EVs have been diluted in 50 ml of PBS 1x and then concentrated up to 2 ml by TFF-Easy and MWCO concentrators 100 K (Millipore). The particle concentration in the final volume has been detected by NTA (Zetaview, Particle Metrix), and compared to 30 ug of EVs diluted in 2 ml of PBS 1X. TFF-Easy allowed a recovery of approximately the 83% of the particles in solution.

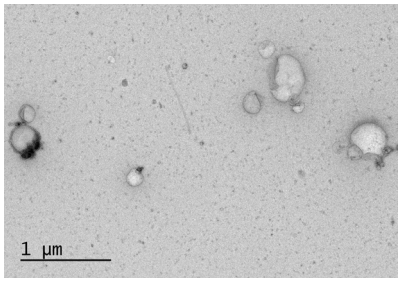
TFF-Easy allows to change the EV buffer without dialysis process

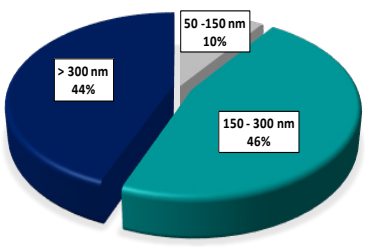
Dialysis progress	Conductivity (μS/cm)	Particle concentration (particle number/ml)
EVs in buffer 1 (PBS 1X) 5 ml	15000	$5.8x10^{11}$
1- Removal of buffer 1 by TFF	15000	
2- Injection of buffer 2 in TFF		
3- Removal of buffer 2 and buffer 1 residues	4100	
4- Injection of buffer 2		
5- Removal of buffer 2	624	
6- Injection of buffer 2		
7- Concentration of buffer 2 up to 5 ml	621	$4.9x10^{11}$

2. Process for EV dialysis with TFF-Easy.

TFF-Easy allows to dialyze EV preparation. In the process described in figure 2 we performed the EV dialysis from buffer 1 (PBS1x) to buffer 2 (NaCl 100 mM).
The TFF-Easy allows the complete removal of buffer 1, without affecting the EV concentration.

TFF-MV: Large EVs isolation, with minimal loss of small EVs

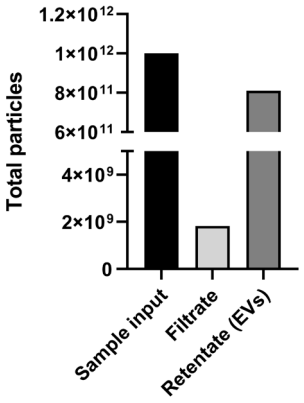




3. EM image and particle size distribution of EVs isolated with TFF-MV

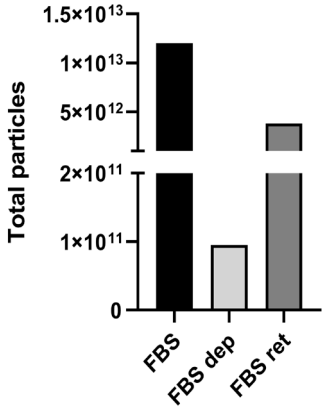
Currently, large MV are isolated or removed from small EVs by centrifugation (10000 g for 30 minutes), which also causes a massive loss of small vesicles. Moreover, different equipment (centrifuges, rotor angle, etc.) has impact on the final results. TFF-MV allows the removal of MV, provides their concentration and purification in one single step, skiping the centrifugation. The isolated MVs are pure and suitable for multiple analyses.

TFF-EVs: fast and scalable system for EV purification from CCM, biofluid, plant extracts



1x10^{12} particles of purified EVs (HBM-PEU-100) were diluted in 20 ml of PBS 1x and then injected into TFF-EVs Small. Retentate containing EVs was recovered in 5 ml of PBS 1x. The particle content of the filtrate and retentate were analyzed by NTA (Zetaview, Particle Metrix).

TFF-EVs: fast and scalable system for EV purification from CCM, biofluid, plant extracts



TFF-EVs was used to depleat the FBS from EVs of bovine origine. 50 ml of raw FBS were filtered through TFF-EVs, the filtrate contained the deplated FBS, whereas bovine EVs were recovered from the retentate in 10 ml PBS 1x buffer. All the three fractions were analyzed by NTA ((Zetaview, Particle Metrix). EV depleted FBS contains only the 1% of the total particles detected in the raw FBS.

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Extracellular Vesicle isolation

Characteristics

- Filter membrane in polyethersulfone.
- MWCO: 100 kDa.
- Concentration volume: from 2.5 ml to 0.050 ml.
- Reverse ultrafiltration (opposite direction to the centrifugal force).

Applications

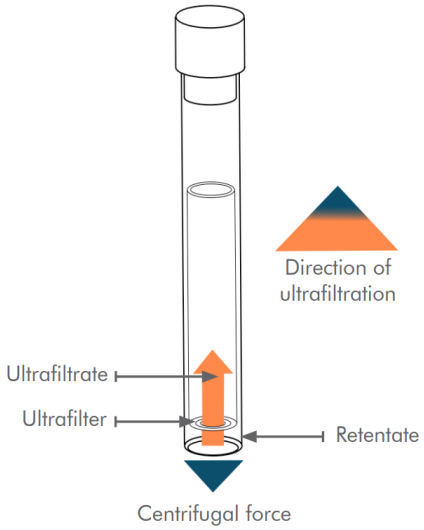
- Concentration of samples containing EVs or nanopartilces.
- Concentration of small volumes of diluted fluids.
- EV dialysis for changing buffer conditions.
- Removal of contaminants and small molecules (unbound dyes).

Advantages

- Non-stick.
- Easy recovery of concentrated material.
- Suitable for multiple washing.

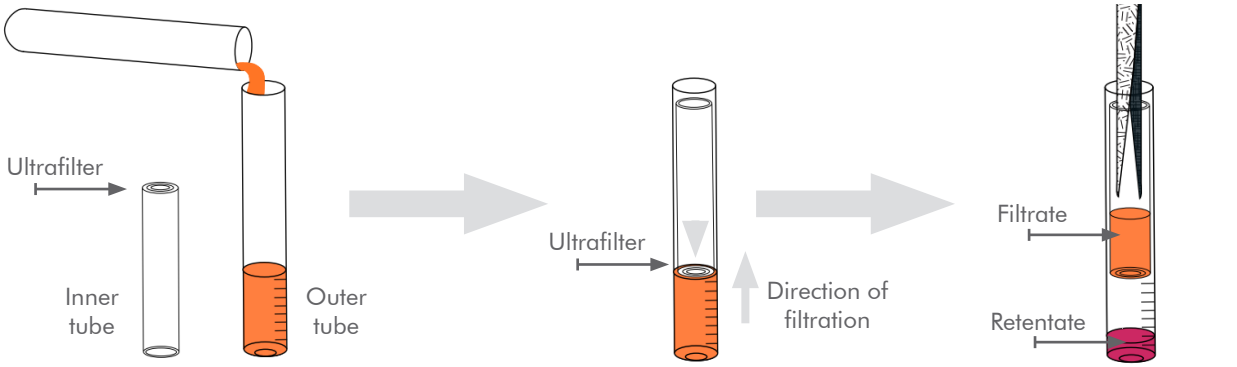
EV-Spinner: ultrafiltration concentrator

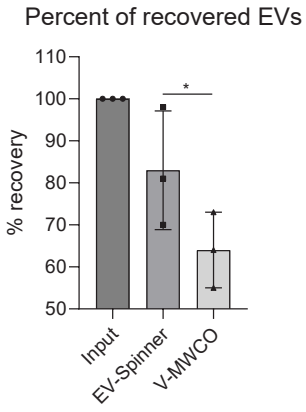
EV-Spinner is a 2.5 ml non-stick ultrafiltration (UF) concentrator to enhance the recovery of extracellular vesicle (EVs) during the concentration or buffer exchange step. The ultrafiltration works in the opposite direction to the centrifugal force, providing higher particle recovery. The low protein binding membrane (polyether-sulphone) reduces the EV loss, compared to V-shape concetrators, and the reverse design of the EV-spinner ensures that the filter does not clog.



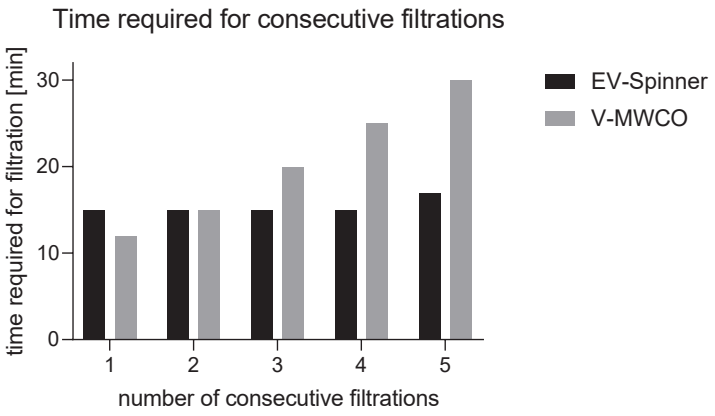
Cat. Code	Description
EV-Spinner ultrafiltration concentrator	
HBM-EVS-24	EV-Spinner 100 kDa MWCO concentrators, 24 pieces
HBM-EVS-48	EV-Spinner 100 kDa MWCO concentrators, 48 pieces

Reverse ultrafiltration for maximum recovery of extracellular vesicles and nanoparticles





Up to 98% of EV recovery was observed with 2.5 ml of EV solution concentrated up to 0.25 ml. Symbols are biological repeats, bars indicate means and error-bars are SDs.



EV-Spinner allows consecutive concentrations (from 2.5 ml up to 0.25 ml) with minimun clogging of the filter. The clogging of the filter was measured by the time necessary for concetrating a solution of EVs from 2.5 ml up to 0.25 ml.

Extracellular Vesicle isolation

Characteristics

- New gel matrix for EV purity improvement.
- Purification up to 20 ml volume of fluid.

PURE-EVs Size Exclusion Chromatography Columns

Size Exclusion Chromatography (SEC) is an efficient method for isolating and purifying Extracellular Vesicles (EVs) from different fluids, not affecting the original shape and functionality of the vesicles. We have developed a set of SEC columns which allow the EV purification from small (100 µl) and large volumes (up to 20 ml) of fluids. The EV purification process with PURE-EV columns is very fast, taking approximately 15 minutes of time.

Applications

- Extracellular vesicles isolation from cell media, biofluids and plant extracts.
- Purification of EVs from contaminants.
- Dye excess removal post EV labeling process.

Cat. Code	Volume	Columns
PURE-EV: Size Exclusion Chromatography columns		
HBM-PEV-5	500 µl - 2 ml	5 Columns
HBM-PEV-10	500 µl - 2 ml	10 Columns
miniPURE-EV: Size Exclusion Chromatography columns		
HBM-mPEV-10	100 µl - 500 µl	10 Columns
HBM-mPEV-20	100 µl - 500 µl	20 Columns
maxiPURE-EV: Size Exclusion Chromatography columns		
HBM-mxPEV-3	1 ml - 20 ml	3 Columns
HBM-mxPEV-6	1 ml - 20 ml	6 Columns

Advantages

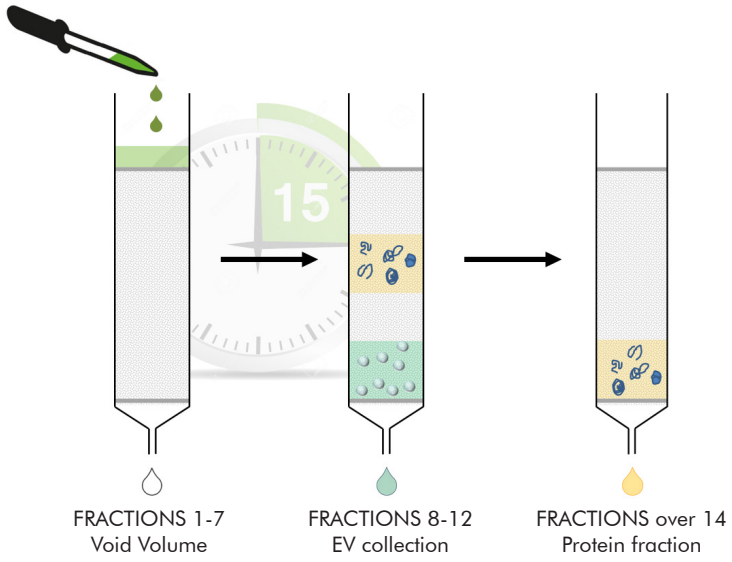
- Easy and fast protocol (turnaround time approximately 15 minutes).
- Isolate EVs from small sample volumes.
- Reusable up to 5 times.
- Long term stability at 4°C.

Maxi-PURE-EVs
Code: HBM-mxPEV
Volume: 2 - 20 ml

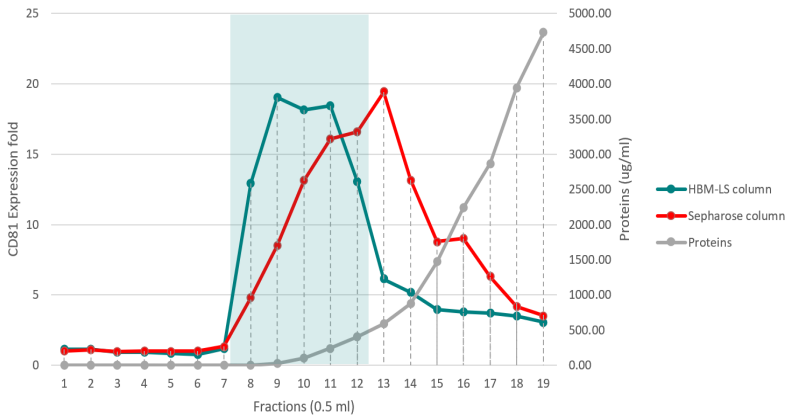
PURE-EVs
Code: HBM-PEV
Volume: 0.5 - 2 ml

Mini-PURE-EVs
Code: HBM-PEV
Volume: 0.1 - 0.5 ml

PURE-EVs: isolation of highly pure extracellular vesicles in approximately 15 minutes

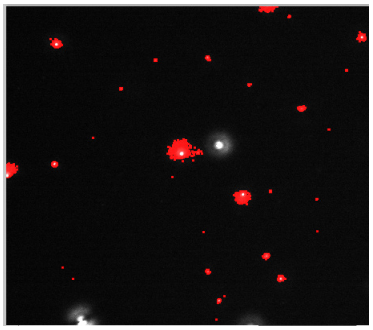


PURE-EVs column was rinsed with 1 ml of cell conditioned media from HCT116 cells, preconcentrated with TFF-Easy 10 fold. 24 fractions (500 µl each one) have been collected and analyzed by ELISA ExoTEST™ assay (see section 3, ExoTEST quantification kit) and by BCA test for determining EVs and total protein content. Results were compared with a column filled with Sepharose CL2B (GE Healthcare). EVs are eluted in fractions 8 - 12, whereas the peak corresponding to protein fraction starts from fraction 14 (Fig 4).

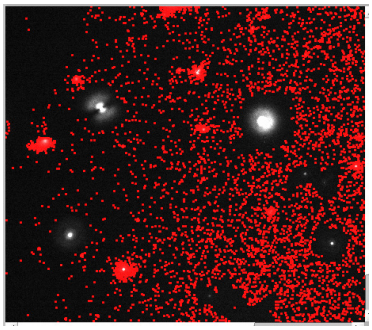


4. EV purification with PURE-EVs column (green line) vs column filled with Sepharose CL2B

mini-PURE-EVs: optimal method for removing the dye excess post EV labeling



5. Dye excess removed by mini-PURE-EVs



6. Dye excess not removed

10 µg of purified EVs from HCT116 cells were labeled by the membrane dye Cell Mask Green. The excess of the dye has been removed from the EV preparation using a mini-PURE-EVs column. The background removal has been detected by NTA with Zetaview (Particle Metrix) (Fig 5,6).

Extracellular Vesicle isolation

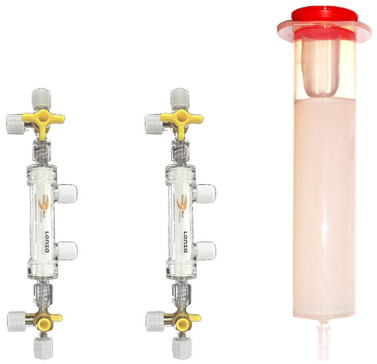
Applications

- Separation and purification of both large (> 150 nm) and small EVs.
- EV concentration and buffere exchange.
- Suitable for conditioned media and diluted biofluids (Urine).

PURE-EVs COMBO kits: EV purification from diluted fluids

PURE-EVs PLUS and PURE-EVs COMPLETE are kits which combine the ability of the TFF filters to concentrate diluted fluids to the capacity of the PURE-EVs columns to purify EVs from circulating proteins. The Combo kits are the perfect solution for people who isolate EVs from fluids as cell conditioned media or urine and want to obtain a high recovery and separation of small and large vesicles.

PURE-EVs COMPLETE: Double TFF and SEC for scalable and reproducible EV isolation and size fractionation from diluted fluids.



TFF-MV TFF-Easy Maxi-PURE-EVs

PURE-EVs PLUS: TFF-Easy and SEC combination for EV isolation and size fractionation.

TFF-Easy Maxi-PURE-EVs

Cat. Code	Volume	Columns
PURE-EV COMPLETE: Complete EV purification and fractionation		
HBM-PEV-5	500 µl - 2 ml	5 Columns
HBM-PEV-10	500 µl - 2 ml	10 Columns
PURE-EV PLUS:		
HBM-mPEV-10	100 µl - 500 µl	10 Columns
HBM-mPEV-20	100 µl - 500 µl	20 Columns

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Extracellular Vesicle isolation

Immunoaffinity isolation of Extracellular Vesicles

Addressing the EV heterogenity is becoming an important issue, in particular for the different roles that Extracellular Vesicles have in pathological processes as cancer, infection, or neurodegenerative diseases.

HBM-LS provides pre-coated ELISA Immunoplates and Latex or Magnetic Immunobeads for the capture and enrichment of total or specific EV-subpopulations.

Applications

- Multiple profiling of EV markers from a single sample or screening of a large number of samples.
- EV capture and quantification from human biofluids (plasma, serum, urine, saliva).
- Suitable for nucleic acid extraction from immunocaptured EVs.

Immunoplates for EV capture and isolation

HBM-LS Immunoplates are 96 multiwell plates covalently pre-coated with specific EV-binding antibodies allowing the capture and isolation of vesicles from different sources (cell supernatant, human plasma, serum, urine and saliva). We developed different types of plates for capturing the total or for enriching specific EV subpopulations (tumoral, neural, glial derived). Plates are blocked and stabilized for long-term storage.

Advantages

- Ready to use.
- Long term storage (up to 2 years).
- No EV pre-purification required
- Small amount of sample required (100 µl per well).
- Flexibility in designing a multiplexing assay.
- Open platform for customized coating solutions.

Immunoplates for Total EV capture and isolation

Cat. Code	Immunoplate	Antibody	Recommended for
ELISA Immunoplate for CD9 positive Extracellular Vesicles capture			
HBM-POS-CC/T1	Transparent	Anti Human CD9 Mouse Monoclonal	Human Plasma, Serum, Urine
HBM-POS-CC/W1	White		
ELISA Immunoplate for CD63 positive Extracellular Vesicles capture			
HBM-POC-CC/T1	Transparent	Anti Human CD63 Mouse Monoclonal	Cell conditioned media, Biofluids
HBM-POC-CC/W1	White		
Custommade Immunoplate			
Plates can be covalentely coated with EV binding antibodies, choosen from our antibody list or sent by customers. Plates are available in transparent and white format.			
Storage conditions			
Unopened: 2 years, stored at 4°C. Opened: 6 month stored at 4°C			
Material amount			
100 µl of sample per well. Whole human plasma and serum can be loaded for vesicle capture. Using urine and cell media, it is recommended to concentrate the sample 10 folds, before loading the sample on the plate.			
Packaging information			
Immunoplates are individually sealed in an opaque aluminium ziplock bag, compliant to pharmaceutical regulations. Easy to open and reseal.			

Immunoplates for enrichment of Tumor-derived EVs

Immunoplates coated with antibodies against TM9SF4 or EpCAM, two proteins widely expressed in tumor tissues and in tumor-derived EVs.

TM9SF4: TM9SF4 is a membrane protein involved in the activation of V-ATPases in conditions of elevated intracellular concentration of H+ as a consequence of elevated fermentation of sugars (Warburg effect). Ref: Lozupone F. et al. 2015

EpCAM: EpCAM is a transmembrane glycoprotein highly expressed in rapidly growing epithelial tumors. It plays an important role in localization of EVs in numerous physiological and phatological processes. Ref: Jiang L. et al. 2017; Yu L. et al. 2013

Cat. Code	Immunoplate	Antibody	Recommended for
ELISA Immunoplate for TM9SF4 Positive Extracellular Vesicles capture			
HBM-PTF-CC/T1	Transparent	Anti Human TM9SF4 Mouse Monoclonal	Human Plasma, Serum
HBM-PTF-CC/W1	White		
ELISA Immunoplate for EpCAM Positive Extracellular Vesicles capture			
HBM-PTE-CC/T1	Transparent	Anti Human EpCAM Mouse Monoclonal	Human Plasma, Serum
HBM-PTE-CC/W1	White		

Immunoplates for enrichment of Neural and Glial EVs

Immunoplates coated with antibodies against EV surface antigens and indicative of neurological or glial origin.

L1CAM: L1CAM is a neural cell adhesion molecule, implicated in cell migration, adhesion and neuronal differentiation. L1CAM is highly expressed in EVs from neural origin and cna be used for enrichment of neural derived EV subpopulation. Ref: Mustapic et al. 2017

PLP1: PLP1is the major myelin protein from the central nervous system and plays an importan role in the formation and the maintenance of the myelin structure. EVs derived from glial cells are characterizec by the presence of high levels of PLP1 protein.
Ref: Frühbeis et al. 2012

Cat. Code	Immunoplate	Antibody	Recommended for
ELISA Immunoplate for L1CAM Positive Extracellular Vesicles capture			
HBM-PNF-CC/T1	Transparent	Anti Human L1CAM-Mouse Monoclonal	Human Plasma, Serum, Cell media
HBM-PNF-CC/W1	White		
ELISA Immunoplate for PLP1 Positive Extracellular Vesicles capture			
HBM-PGF-CC/T1	Transparent	Anti Human PLP1 Mouse Monoclonal	Human Plasma, Serum, Cell media
HBM-PGF-CC/W1	White		

Applications

- Capture and enrichment of Tumor derived EVs subpopulations.
- Suitable for nucleic acid extraction from immunocaptured EVs.
- Profiling of cancer related biomarkers.

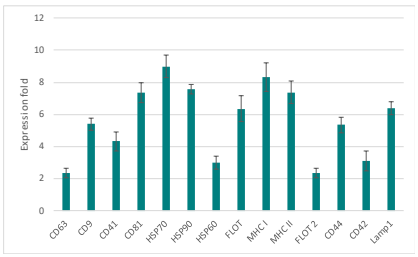
Advantages

- Ready to use.
- Long term storage (up to 2 years).
- No EV pre-purification required
- Small amount of sample required (100 µl per well).
- Open platform for customized coating solutions.

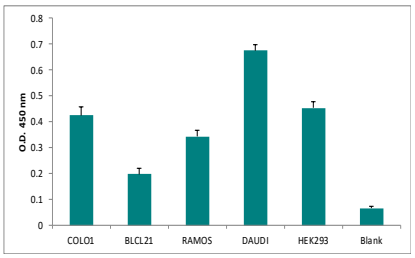
Extracellular Vesicle isolation

Immunoplates allow EV phenotyping without vesicle pre-purification steps

ELISA Immunoplates can be used for quantitative and qualitative analysis of EV-associated proteins. The plate is able to capture EVs from raw biologic material (plasma, serum, cell medium, etc.). No significant cross-reactivity is observed with soluble antigens.



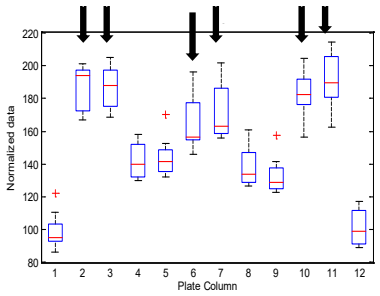
9. EV associated biomarkers analysis in a healthy donor's plasma sample.

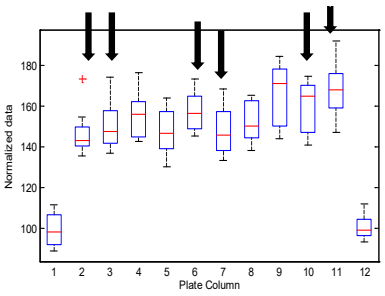


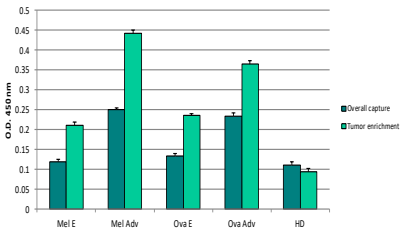
10. CD63 profiling of different cell derived EVs.

Enrichment of Tumor-derived Extracellular Vesicles

Immunoplates for tumor-derived EV enrichment (TM9SF4 coated) are able to distinguish cancer patients (black arrows) from healthy controls. The enrichment of tumor-derived EVs from cancer patient (Melanoma, Ovary) is detectable when the TM9SF4 coated plate is compared with a plate coated with CD9 (fig11).



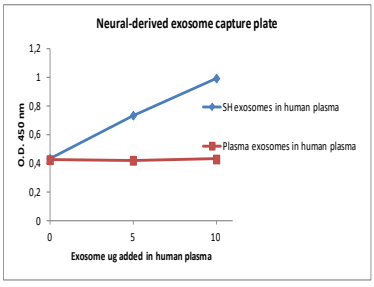




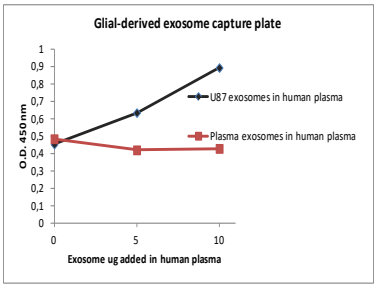
11. Enrichment in Tumor-derived EVs in early and late stage melanoma (Mel E; Mel ADV) and ovary carcinoma (Ova E, Ova ADV).

Enrichment of Neural or Glial derived Extracellular Vesicles

Immunocapture enrichment of neural- and glial-derived EVs purified from SK-N-SH and U87 cell lines and spiked in human plasma. Comparison was done with purified plasma EVs spiked in human plasma (fig 12 and fig 13).



12. Enrichemnt of SK-N-SH derived EVs spiked in human plasma from healthy donors using HBM-PNF



13. Enrichmentof U87 derived EVs spiked in human plasma from healthy donors using HBM-PGF

Immunobeads for Total EV isolation

Latex or Magnetic immunobeads are covalently coupled with antibodies against common EV surface antigens (CD9, CD63). They allow capturing EV from human biofluids (tested for plasma, serum and urine) and cell culture media without the necessity of pre-purification steps. The kit includes a Beads Elution buffer, for detaching captured EVs from antibodies and a Regeneration buffer, for regenerating the beads that can be reused ones more. Beads are sold in package of 10 reactions and are available in 2 sizes (0.4 and 1 micron diameter).

Cat. Code	Bead diameter	Antibody	Recommended for
Immunobeads for CD9 Positive Extracellular Vesicles capture			
HBM-BOLF-CC/10-04	0.4 micron	Anti Human CD9 Mouse Monoclonal	EV pheno/genotyping
HBM-BOLF-CC/10-1	1 micron		FACS analysis
Immunobeads for CD63 Positive Extracellular Vesicles capture			
HBM-BOLC-CC/10-04	0.4 micron	Anti Human CD63 Mouse Monoclonal	EV pheno/genotyping
HBM-BOLC-CC/10-1	1 micron		FACS analysis
Immunobeads for Mouse Extracellular Vesicles capture (CD9 Positive)			
HBM-BMLF-CC/10-04	0.4 micron	Anti Mouse CD9 Mouse Monoclonal	EV pheno/genotyping
HBM-BMLF-CC/10-1	1 micron		FACS analysis
Custommade Latex or Magnetic Immunobeads			
Beads can be covalentely coated with EV binding antibodies, choosen from our antibody list or sent by customers. Magnetic or Latex beads are available.			
Storage Condition			
Store the immunobeads and buffers at 4 - 8° C.			
Conditions required			
Recommended starting volume from 0.1 ml - 0.5 ml of plasma, from 0.5 ml to 1 ml of serum. Concentrated (10X) urine and cell culture medium samples are recommended prior capture according to our suggested protocol (see page 21, TFF-Easy).			

Applications

- Total EV isolation from cell culture media, human or mouse biofluids (tested for plasma, serum, urine).
- Total EV isolation from mouse biofluids (tested for plasma and serum).
- Downstream marker profiling.
- Nucleic acids extraction
- EV elution from immunobeads

Advantages

- Ready to use.
- Small sample volume of biofluid or cell culture medium.
- No ultracentrifugation or other methods for vesicle purification required.
- Supplied with buffer for EV elution from beads.
- Immunobeads can be regenerated with Beads Regeneration Buffer and reused.

2

Extracellular Vesicle isolation

Applications

- Capture and enrichment of human EV subpopulation (tumor-derived).
- Downstream EV marker profiling.
- Nucleic acids extraction
- EV elution from immunobeads

Immunobeads for Tumor-derived EV capture

Latex or Magnetic immunobeads are covalently coupled with antibodies against EV surface antigens (TM9SF4 or EpCAM) associated with pathological conditions (cancer). They allow to pull down tumor-derived EV from human biofluids, thus providing a potential new platform for the research in circulating tumor biomarker.

Cat. Code	Bead diameter	Antibody	Recommended for
Immunobeads for TM9SF4 Positive Extracellular Vesicles capture			
HBM-BTLF-CC/10-04	0.4 micron	Anti Human TM9SF4 Rabbit polyclonal	EV pheno/genotyping
HBM-BTLF-CC/10-1	1 micron		FACS analysis
Immunobeads for EpCAM Positive Extracellular Vesicles capture			
HBM-BTLE-CC/10-04	0.4 micron	Anti Human EpCAM Mouse Monoclonal	EV pheno/genotyping
HBM-BTLE-CC/10-1	1 micron		FACS analysis
Packaging information			
Immunobeads (10 reactions) are supplied with Exosome Elution Buffer, for eluting intact exosomes from beads and with Bead Regeneration Buffer, for regenerating immunobeads that can be reused twice more.			

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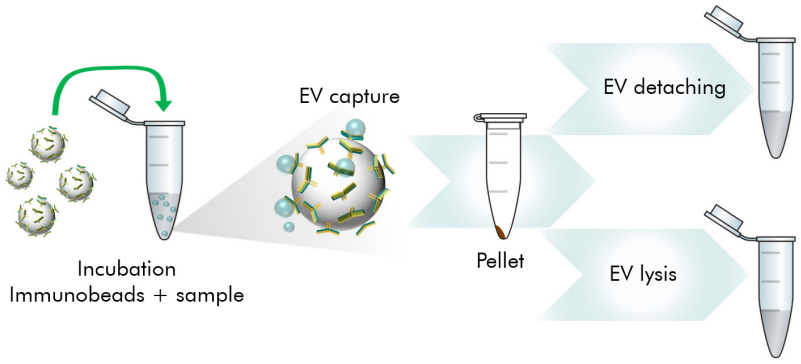
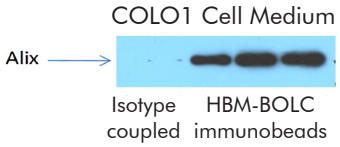
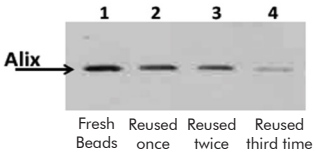
Immunobeads allow EVs capture and multiple downstraem analyses



Following incubation, beads can be recovered by centrifugation, resuspended in Laemmli buffer for SDS-PAGE and western blotting analysis (fig 14, 15) or in appropriate lysis buffer for nucleic acid analysis (fig 16). Alternatively, the vesicles can be eluted from the beads with the Elution Buffer and used for downstream applications such as ELISA or NTA. Eluted beads can be regenerated with Bead Regeneration Buffer and reused for capturing exosomes twice more (fig 15).

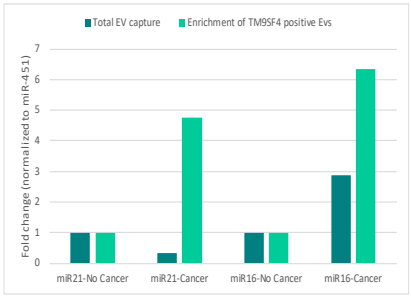


14. Alix expression by western blotting of exosomes captured on HBM-BOLC immunobeads from COLO1 cell supernatant vs isotype coupled beads.

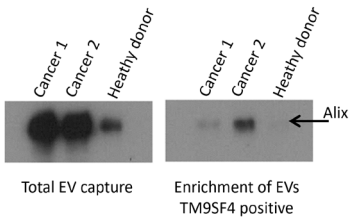


15. Western Blotting analysis of immunocaptured exosomes on beads.

TM9SF4 coated immunobeads enrich Tumor-derived EVs in cancer patient



16. Enrichment of miR21 and miR16 in cancer when TM9SF4 coated beads are used



17. Comparison of detection in WB of total and TM9SF4 positive EVs

Latex or Magnetic Immunobeads can be used for capturing EVs from raw biofluids, followed by RNA isolation. The enrichment of miRNA cancer associated (miR16 and miR21) is highly detectable when Immunobeads for capture of Tumor-derived EVs (HBM-BTF; TM9SF4 coated) are used.

Extracellular Vesicle isolation

Applications

- Single step isolation of EVs from multiple fluids
- Isolate the overall vesicles population in a sample.
- Isolated EVs are suitable for nucleic acid extraction and profiling.
- Isolated EVs are suitable for protein profiling (WB, ELISA, FACS).

Advantages

- Time and money saving.
- No ultracentrifugation required.
- Easy and fast protocol.
- Isolate EVs from small volumes of sample (as low as 100 μl of plasma).
- Easy to store and ship (4°C).

EXO-Prep: one step EV isolation reagent

EXO-Prep is a fast and convenient method of Extracellular Vesicle isolation from biofluids, cell culture supernatants, plant extracts. Isolation with EXO-Prep is based on chemical precipitation. Samples are incubated with EXO-Prep solution on ice so that EVs will precipitate following centrifugation. The obtained pellet can be resuspended in PBS 1X or deionized water. The protocol is user-friendly, time-saving (around 1 hour), and does not require capital laboratory equipment. Isolated vesicles are in particular suitable for isolation of nucleic acid associated to EVs.

Cat. Code	Volume	Reactions
EXO-Prep for Exosome Isolation from Plasma and Serum		
HBM-EXP-B5	5 ml	180 reactions Plasma, 80 reactions Serum
EXO-Prep for Exosome Isolation from Cell Media		
HBM-EXP-C25	25 ml	25 reactions
EXO-Prep for Exosome Isolation from Urine		
HBM-EXP-U25	30 ml	25 reactions

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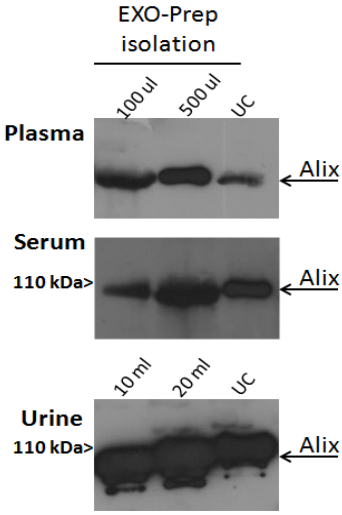
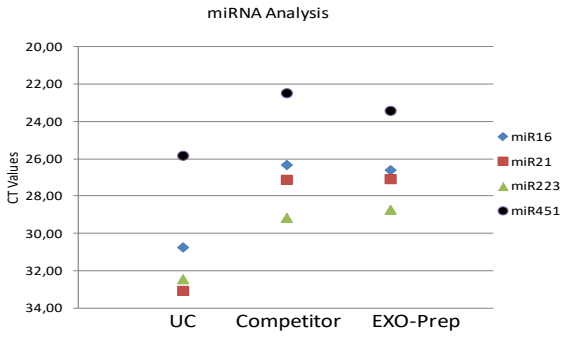
EXO-Prep isolates Extracellular Vesicles in one single step from a small volume of sample



18. WB analysis of Alix in EV lysates

EXO-Prep is able to isolate EVs form very low volume amount. EVs were isolated from 100 µl or 500 µl of human plasma, serum and 10 or 20 ml of human urine. 30 µg of protein lysates have been used for EV marker analysis (Alix) (fig 18). RNAs were extracted from EVs isolated from 500 µl of human plasma and tested for profiling of 4 different miRNA EV associated (fig 19).



19. Profiling of 4 miRNA EV associated.

Chapter 3

Extracellular Vesicle characterization

Summary chapter 3

Extracellular Vesicle characterization

Introduction

EVs research needs of reliable, affordable and optimized tools for quantification and characterization of vesicles in complex biological samples as well as in cellular models. HBM-LS offers the ExoTEST™ and the Exo-FACS kits for quantification and characterization of EV markers by ELISA and FACS, respectively, and the new Enolase Activity Kit, which allows to characterize the different EVs sources by the determination of the enolase activity.

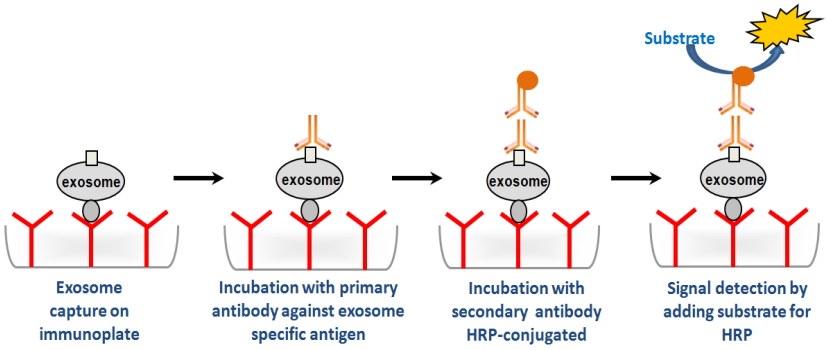
ExoTEST™: ELISA ready-to-use quantification kit

Exo-FACS: EV marker analysis by FACS

EV Enolase: Enolase activity on EVs

ExoTEST™: ELISA ready-to-use quantification kit

ExoTEST™ is a patented double sandwich ELISA assay for quantitative and qualitative analysis of vesicles. In particular, ExoTEST™ is a successful platform for exosome quantification and characterization from small amount of human biological fluids or cell conditioned media. ExoTEST™ enables reliable and precise quantitative measurement and comparison among samples and individual experiments and provides increased sensitivity in detection of EV markers with respect to other analytical methods (i.e FACS, WB).



ExoTEST™ consists of ELISA plates pre-coated with proprietary pan-exosome antibodies enabling specific capture of exosomes from different biological samples. Quantification and characterization of exosomal proteins is subsequently performed using appropriate detection antibodies against exosome surface antigens. Lyophilized Exosome Standards, characterized for protein content and particle number (NTA) allow the quantification of unknown sample by a standard calibration curve.

Kit components	Description
Immunoplate	96 well (12 strips x 8 wells) precoated with specific exosome capturing antibody.
Lyophilized Exosome Standards	Exosome Standards from human plasma, serum, urine, saliva or cell medium for calibration curve.
Antibodies for exosome marker detection	Primary anti-human CD9 antibody (HBM proprietary) and secondary antibody HRP conjugated for exosome detection.
Buffers	Sample buffer for antibody dilution and incubation. Washing buffer for washing ELISA plate.
Reagents	Reagents for signal detection.

Characteristics

- Starting material: 100 µl of biological sample. Whole plasma and serum can be directly loaded on the plate. Concentrate 10 folds urine or cell media prior plate loading.
- The detection limit of the assay is lower than 0.35 µg of EVs.
- Kit contains Lyophilized EVs for assay calibration.

Applications

- Exosome capture and quantification from human biofluids and cell culture media.
- Comprehensive exosome profiling.
- Pre-clinical research on non-invasive biomarkers for detection and monitoring of a number of pathological conditions (inflammation, cancer, neurodegeneration, etc).

Advantages

- Ready to use.
- No initial exosome purification required.
- User friendly and suitable for multiple marker analyses.
- Available in TEST format (limited to 3 ELISA strips, 24 wells).

Extracellular Vesicle characterization

ExoTEST™: ELISA ready-to-use quantification kit

HBM-LS offers different types of ExoTEST™ kits for quantification of Extracellular Vesicle population from human biofluids (plasma, urine, serum) and from cell culture supernatants. Furthermore, ExoTEST™ it is available for Tumor-derived EVs Enrichment and quantification.

Cat. Code	Description	Readout
ExoTEST™ for Extracellular Vesicle immunocapture and quantification from human plasma and urine		
HBM-RTK-POF/##	EV detection performed with anti-human CD9 antibody and anti-mouse HRP conjugated.	Colorimetric
ExoTEST™ for Extracellular Vesicle immunocapture and quantification from human serum		
HBM-RTK-POS/##	EV detection performed with anti-human CD9 biotin-conjugated antibody and Streptavidin-HRP.	Colorimetric
ExoTEST™ for Extracellular Vesicle immunocapture and quantification from cell culture media		
HBM-RTK-POC/##	EV detection performed with anti-human CD9 biotin-conjugated antibody and Streptavidin-HRP.	Colorimetric
ExoTEST™ for Tumor-derived Extracellular Vesicle immunocapture and quantification from human plasma		
HBM-RTK-PTF/##	EV detection performed with anti-human CD9 antibody and anti-mouse HRP conjugated.	Colorimetric
Custom made ExoTEST™ for Specific Extracellular Vesicle immunocapture and quantification		
HBM-RTK-CMK	HansaBioMed Life Sciences offers the flexibility of creating and designing your own kit by choosing among a wide variety of reagents available in our catalog. For information contact info@hansabiomed.eu	
Storage condition		
All reagents are shipped with ice packs and can be stored at 4-8° C.		
All kits are also available in TEST format, limited to 3 ELISA strips (24 wells). Cat Code: HBM-TRTK-###		

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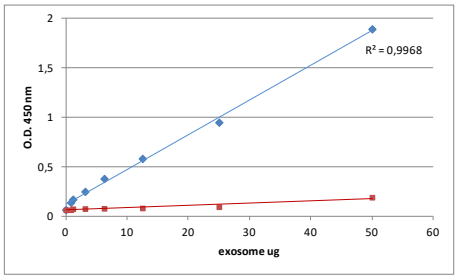
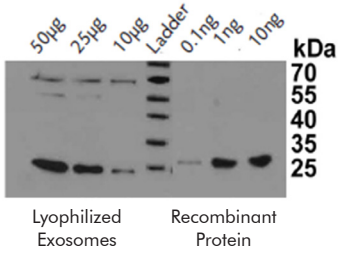
ExoTEST™: High sensitivity in detecting low exosome amount



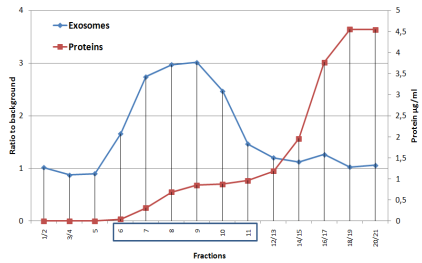
1. CD9 titration (blue line) of healthy donor plasma exosome standards (HBM-PEP100) and comparison with observed background (red line, only secondary antibody).



2. CD9 exosome marker detection by Western Blotting on lyophilized exosomes from human plasma (HBM-PEP100) and recombinant CD9 protein.

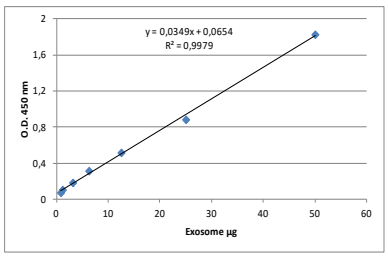
The sensitivity of the ExoTEST™ is higher than western blotting (Figures 1 and 2). 10 µg of lyophilized exosomes are equivalent to 0.1 ng of recombinant exosomal protein: since the standard curve's lower concentration is 0.39 µg of lyophilized exosomes (Fig 1), the sensitivity of our test is around 39 pg of protein equivalent.

ExoTEST™: Highly specific EV binding, without circulating protein contamination

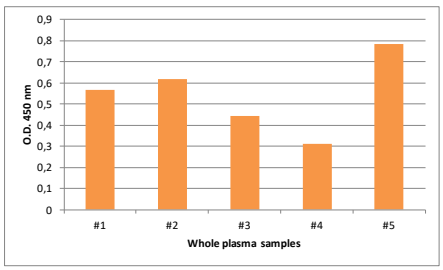


3. Comparison of ExoTEST™ analysis and total protein content (BCA test) of different fractions of human plasma fractioned by SEC column. EVs are eluted in fractions 6-11, whereas the highest yield of plasma proteins appears from fraction 14.

ExoTEST™: Example of Ev quantification from 5



4. Standard curve obtained with Lyophilized Exosome Standards from human plasma healthy donors (HBM-PEP100) with anti-CD9 antibody.



5. CD9 titration of exosomes in 5 different whole plasma from healthy donor.

Example of exosome quantification performed in 5 unknown plasma samples from healthy donors using the ExoTEST™ (HBM-RTK-POF/TP). Following the binding of Lyophilized Standards and unknown samples onto the ELISA plate, test is run according to the kit protocol and exosome detection is performed with anti-CD9 antibody (HBM-LS).
Exosome quantification is finally performed calculating the quantity of exosomes (expressed in µg) in the 5 unknown samples using the equation obtained from the standard curve (Fig 3). The particle number contained in 100 µl of plasma is calculated from quantity of exosomes (expressed in µg) according to the particle concentration (number of particles/ml) indicated in the label of the Lyophilized Exosome Standards (HBM-PEP100, NTA: 3x10^11 particles/ml).

Plasma sample	O.D. 450 nm	EV µg	Particle number in 100 µl of plasma
#1	0,5673	12,869	3,86x10^9
#2	0,6194	14,205	4,26x10^9
#3	0,4425	9,6692	2,90x10^9
#4	0,3100	6,2717	1,88x10^9
#5	0,7853	18,458	5,54x10^9

Extracellular Vesicle characterization

Applications

- Exosome isolation and exosome marker characterization via FACS.
- Comprehensive exosome profiling.

Advantages

- Ready to use.
- No initial exosome purification required.
- Lyophilized Exosome Standards for positive control included.
- User-friendly and suitable for multiple marker analyses.

Kit components

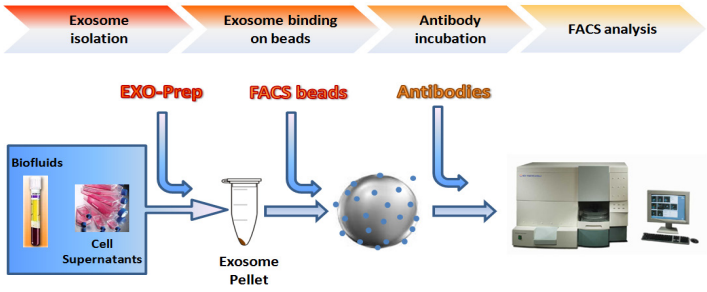
- EXO-Prep for exosome isolation.
- Lyophilized Exosome Standards as positive control.
- Primary antibody for exosome marker detection as positive control.
- Secondary antibody Alexa 488.
- Sample buffer, for antibody incubation.

Storage

All reagents are shipped and must be stored at 4°C.

Exo-FACS: ready-to-use kit for EV FACS analysis

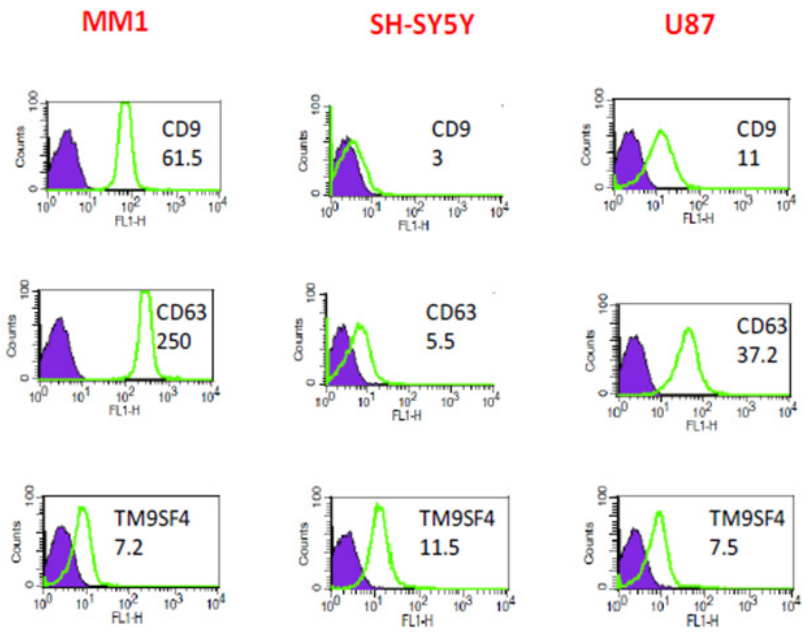
The kit consists of EXO-Prep reagent for exosome isolation, 4 µm beads used for the overall capture of pre-isolated exosomes, lyophilized exosomes from cell culture supernatants or human biological fluids as positive control. The characterization of exosomal proteins (membrane-expressed or internal) is subsequently performed using appropriate detection antibodies against exosome associated antigens.



HBM-LS offers different Exo-FACS kits for staining of EV markers from human biofluids (plasma, urine, serum, saliva) and from cell culture supernatants. Exo-FACS contains reagents for 20 reactions (lyophilized exosomes, beads, antibodies and buffers). Primary antibody included in the kit is against a common exosomal marker (CD9 or CD63) and can be used as a positive control for protein profiling via FACS analysis.

Cat. Code	Description	Lyophilized EV	Detection antibody
Exo-FACS ready to use kits for analysis of exosome marker from human biofluids			
HBM-FACS-PEP	FACS analysis of plasma EVs	HBM-PEP100 1 vial, 100 µg	Anti human CD9
HBM-FACS-PES	FACS analysis of serum EVs	HBM-PES100 1 vial, 100 µg	Anti human CD9
HBM-FACS-PEU	FACS analysis of urine EVs	HBM-PEP100 1 vial, 100 µg	Anti human CD9
HBM-FACS-PESL	FACS analysis of saliva EVs	HBM-PEP100 1 vial, 100 µg	Anti human CD9
Exo-FACS ready to use kits for analysis of exosome marker from cell culture media			
HBM-FACS-C	FACS analysis of cell derived EVs	HBM-###100 * 1 vial, 100 µg	Anti human CD63
* Possibility to choose the lyophilized EVs from the list of Lyophilized EVs from cell media available in the section 1 of this catalog, page 5 and 6.			

Exo-FACS: Exosome protein profiling by Flow Cytometry technique



6. FACS profiling of exosomal markers CD9, CD63 and TM9SF4 in purified exosomes from MM1, SH-SY5Y and U87 cell lines.

Exo-FACS was used for a protein marker profile in exosomes derived from different sources. Exosome binding on FACS-beads was performed by incubation at 4°C overnight. Exosome-bead complex is ready to be labeled with fluorophore-conjugated antibodies for specific exosome markers. In figure 6 is shown a profile of expression of three different exosome markers in exosomes purified from Melanoma (MM1), Neuroblastoma (SH) and Glioblastoma (U87) cell supernatants.

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Shtam, T. A., Samsonov, R. B., Volnitskiy, A. V., Kamyshinsky, R. A., Verlov, N. A., Kniazeva, M. S., ... & Malek, A. V. (2018). Isolation of Extracellular Microvesicles from Cell Culture Medium: Comparative Evaluation of Methods. Biochemistry (Moscow), Supplement Series B: Biomedical Chemistry, 12(2), 167-175.

Extracellular Vesicle characterization

Characteristics

- Starting material: minimum 1x10^8 particles per reaction.
- Kit contains Lyophilized EVs as positive control.
- Fluorimetric and colorimetric readout.

Applications

- EV characterization by funcional properties.
- Determination of the Enolase activity in purified/isolated EVs.
- Determination of the functionality and stability of EVs from cell lines.
- Mechanistic studies of EVs of cancer origin

Advantages

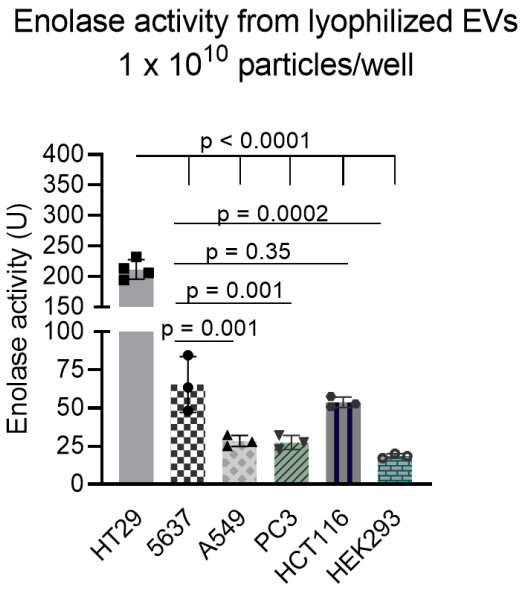
- Ready to use.
- Suitable for measuring enolase activity from fresh, frozen or lyophilized cell-derived EVs

EV-Enolase: Enolase activity on EVs

Enolase (EC 4.2.1.11), also called 2-phospho-D-glycerate hydrolase or 2-phosphoglycerate dehydratase, is a key enzyme in glycolysis. It converts 2-phosphoglycerate to phosphoenolpyruvate (PEP) & also catalyzes the reverse reaction, PEP to 2-phosphoglycerate under anabolic conditions during gluconeogenesis. This enzyme exists in all organisms, which can undergo glycolysis. Enolase activity is easily detectable in extracellular vesicles (EVs) derived from eukaryotic cells and it could be used for evaluating functionality and stability of EVs. Moreover, it's increased activity is associated with tumorigenesis and therefore precise measurement of enolase activity may be of great interest for EV-based tumor diagnosis.

Cat. Code	Description
HBM-K691-EN	Enolase activity on Extracellular Vesicles
Shippment and storage: Kit is shipped at controlled temperature with ice pack. Store the components as indicated in the product datasheet.	

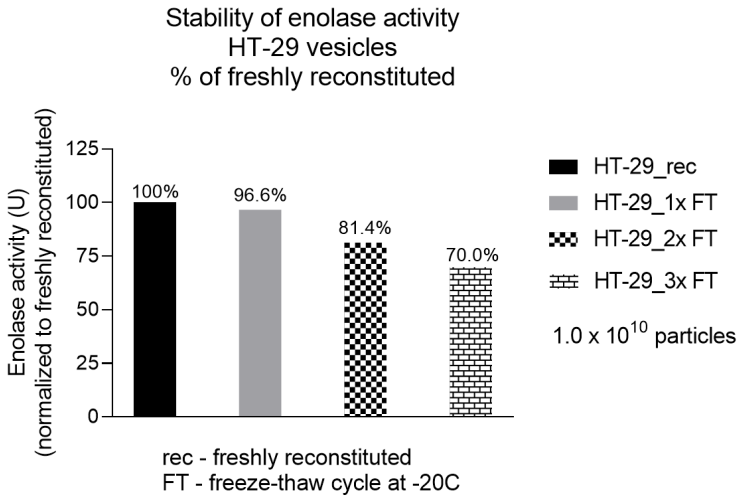
Cancer cell derived EVs show different profile of enolase activity



Enolase activity in EVs isolated from conditioned media of various cell lines. $1x10^{10}$ particles were used and the enolase activity was calculated based on the standard curve. Statistical analyses with one-way ANOVA and Dunnett's multiple comparison test. Symbols are biological repeats, bars indicate means and error-bars are SDs.

7. Enolase activity in EVs purified by SEC from different cancer cells.

Enolase activity is indicative of the EV state



Lyophilized HT-29 EVs were reconstituted in MilliQ water and 1, 2 or 3 freeze-thaw cycles at -20° C were performed. Enolase activity was normalized relative to the freshly reconstituted EVs (rec). One freeze-thaw cycle does not affect the enolase activity compared to the freshly reconstituted sample.

8. Stability of enolase activity in extracellular vesicles (EVs) after lyophilization and freezing/thawing cycles.

Chapter 4

Extracellular Vesicle RNA isolation

Summary chapter 4

Extracellular Vesicle RNA isolation

Applications

- Direct capture and exosome RNA extraction from human biofluids and cell culture media without initial exosome purification step.
- Simultaneous miRNA and mRNA profiling (qRT-PCR, RT-PCR, microarray).

Introduction

EVs shuttle functional RNA molecules in the target cell and EV-derived miRNAs, in particular pathogenic miRNAs, might be exploited as novel therapeutic targets or disease biomarkers , including cancer. miRNAs seem to play critical roles as transcriptional and post-transcriptional regulators of epigenetic mechanisms and cell processes and have been linked to the etiology, progression and prognosis of cancer. Similar miRNA expression patterns between tumor tissue samples and circulating exosomes have been observed.

Advantages

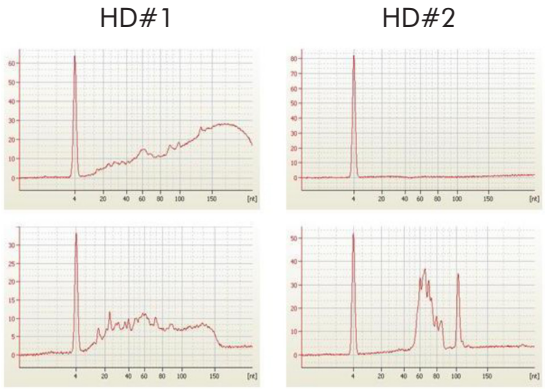
- High yield of total RNA (including small RNAs).
- Fast and user-friendly protocol.
- Small starting amount of sample (less than 1 ml).

EV-totalRNA: RNA-EV associated isolation kit

Kit allows RNA extraction from exosomes pre-isolated with different methods (ultracentrifugation, chemical precipitation, immunocapture, size-chromatography etc.)

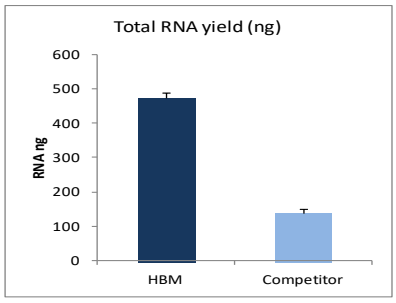
Cat. Code	Description	Size
EV-totalRNA: RNA-EV associated isolation kit		
HBM-RNA-B25	RNA extraction from pre-isolated EVs	25 reactions
Compatible with EVs isolated via ultracentrifuge, chemical precipitation, size chromatography, immunocapture etc.		

High quality and yield of EV-associated RNAs from small volumes of sample

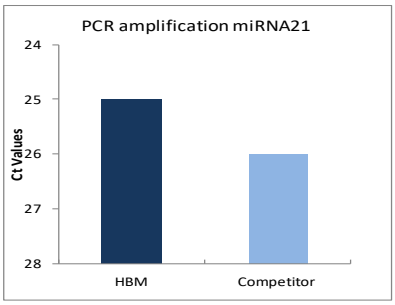


1. Electropherograms of small RNA extracted with HBM EXO-Total RNA kit and Competitor (Agilent 2100 Bioanalyzer)

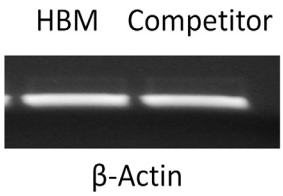
Efficiency of EV-TotalRNA kit was tested vs a competitor kit for RNA extraction from plasma-derived EVs. Extraction of total exosome RNA was performed from 100 µl of healthy donor plasma (HD #1 an #2) either with Competitor Kit and the EXO-TotalRNA Kit (HBM-LS). RNA quality was evaluated by electopherogram (Fig 1) with Small RNA microfluidic chips (Agilent 2100 Bioanalyzer). RNA yield was quantified by Nanodrop (Fig 2) and extracted RNA was subsequently retrotranscribed using the miScript II RT kit (Qiagen). miR-21 and b-actin markers were amplified by qPCR (Fig 3 and 4).



2. Nanodrop quantification of total RNA yield



3. miRNA 21 amplification by qPCR



4. β-Actin amplification by PCR

Chapter 5

Antibodies for EV research

Summary chapter 5

Antibodies for EV research

Introduction

Antibodies are an essential tool for basic research, development of diagnostic tests and therapeutics for human disease. Extracellular vesicles (EVs), including exosomes, express antigens with 3D conformations and/or post-translational modifications that often differ from the cellular counterpart. For this reason, most of the antibodies currently available on the market fail to recognize exosome-associated antigens with sufficient sensitivity and specificity. HBM-LS has validated a list of polyclonal and monoclonal antibodies against common (CD63, CD81, ALIX) and disease- specific (cancer and neurodegenerative diseases) exosomal markers.

Antibodies for common and disease-associated EV markers

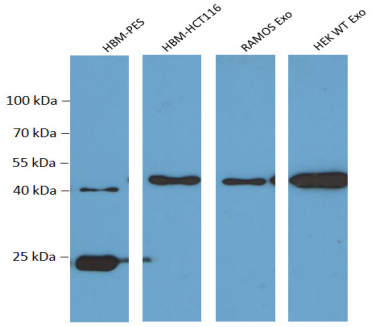
Cat. Code	Antibody	Type	Applicartions
Antibodies for common EV Markers			
HBM-TSG101-###	Anti human TSG101	Mouse monoclonal unconjugated	WB, IHC
HBM-CD9-###	Anti human CD9	Mouse monoclonal unconjugated	WB, FACS, ELISA, IP, IHC
HBM-CD9B-###	Anti human CD9	Mouse monoclonal biotin conjugated	WB,, ELISA, IP
HBM-CD63-###	Anti human CD63	Mouse monoclonal unconjugated	WB, FACS, ELISA, IP, IHC
HBM-CD41-EM1-###	Anti human CD41	Mouse monoclonal unconjugated	FACS, ELISA
HBM-CD81-EM4-###	Anti human CD81	Mouse monoclonal unconjugated	WB, FACS, ELISA, IP, IHC
HBM-ALIX-###	Anti human Alix	Mouse monoclonal unconjugated	WB, FACS, IF
HBM-FLOT-###	Anti human Flotillin	Rabbit polyclonal unconjugated	WB,FACS, ELISA
HBM-RAB5-PR1-###	Anti human RAB5	Rabbit polyclonal unconjugated	WB, FACS, ELISA, IP
HBM-CD9M-###	Anti mouse CD9	Mouse monoclonal unconjugated	WB, FACS, ELISA, IP
Antibodies for EV-associated disease markers			
HBM-HSP70-SR1-###	Anti human HSP70	Rabbit polyclonal unconjugated	WB, FACS, ELISA
HBM-SF4-PR2-###	Anti human TM9SF4	Rabbit polyclonal unconjugated	WB, IP
HBM-CD44-EM1-###	Anti human CD44	Mouse monoclonal unconjugated	WB, ELISA, IP
HBM-CAV1-D4-###	Anti human Caveolin 1	Mouse monoclonal unconjugated	WB, FACS

List of abbreviations:

ELISA: Enzyme-Linked Immunosorbent Assay
FACS: Fluorescent-Activated Cell Sorting
IHC: Immunohistochemistry
IP: Immunoprecipitation
IF: Immunofluorescence
WB: Western Blotting

Anti human TSG101 antibody

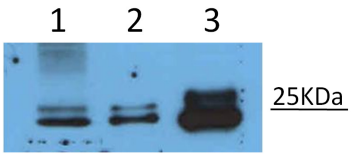
Description	TSG101, a 46 kDa protein, is the product of a recently identified Tumor Susceptibility Gene whose inactivation in mouse fibroblasts results in cell transformation and the ability of those cells to form tumors in nude mice. TSG101 is highly expressed internally in EVs and it is considered one of the common markers for exosome detection.
Cat Num/Amount	HBM-TSG101-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, IHC



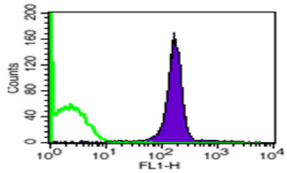
Western Blotting
Detection of TSG101 in 20 µg of Exosome Standards from:
1-HBM-PES: healthy human serum
2-HBM-HCT116: HCT116 cell culture medium
3-RAMOS Exo: exosomes purified from RAMOS cell medium
4- HEK293 WT Exo: exosomes purified from HEK293 wild type cell medium.

Anti human CD9 antibody

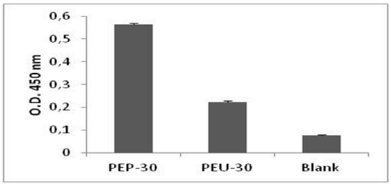
Description	Anti-CD9 recognizes a human 24-kiloDalton (kDa) single-chain cell-surface glycoprotein (p24) belonging to the tetraspanin family. CD9 has a very broad tissue distribution and is abundant on exosome membranes. HBM-LS offers monoclonal anti-CD9 antibodies, unconjugated or biotin conjugated, recognizing the specific antigen on both cell lines and extracellular vesicles.
Cat Num/Amount	HBM-CD9-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP, IHC
References	- Rampini, S., et al. "Micromagnet arrays for on-chip focusing, switching, and separation of superparamagnetic beads and single cells." Lab on a Chip 15.16 (2015): 3370-3379. - Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



Western Blotting
1- MM1 (melanoma cell) lysate (20 µg)
2- MM1 cells purified exosomes (20 µg)
3-Plasma healthy donors purified exosomes (20 µg)



FACS
CD9 staining of exosomes purified from human plasma

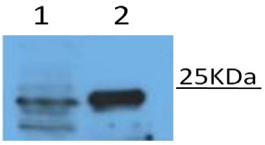


ELISA
CD9 detection in purified exosomes from human plasma (PEP) and urine (PEU), 30 µg

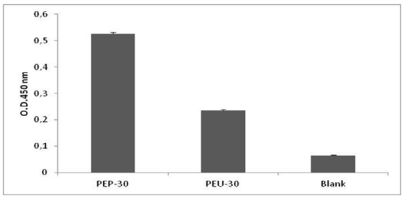
Antibodies for EV research

Anti human CD9 antibody biotin conjugated

Description	Anti-CD9 recognizes a human 24-kiloDalton (kDa) single-chain cell-surface glycoprotein (p24) belonging to the tetraspanin family. CD9 has a very broad tissue distribution and is abundant on exosome membranes. HBM offers two monoclonal anti-CD9 antibodies, unconjugated or biotin conjugated, recognizing the specific antigen on both cell lines and extracellular vesicles. HBM antibodies are compatible with ELISA, WB and flow cytometry applications.
Cat Num/Amount	HBM-CD9B-100 (100 µg)
Type	Mouse monoclonal biotin conjugated
Reactivity	Human
Applications	WB, ELISA, IP
References	- Rampini, S., et al. "Micromagnet arrays for on-chip focusing, switching, and separation of superparamagnetic beads and single cells." Lab on a Chip 15.16 (2015): 3370-3379. - Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



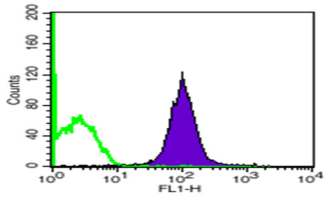
Western blotting
1- COLO1 cell lysates (20 µg)
2- CD9 detection in plasma purified exosomes (20 µg)



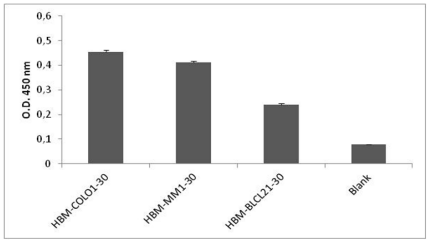
ELISA
CD9 detection in purified exosomes from human plasma (PEP) and urine (PEU), 30 µg

Anti human CD63 antibody

Description	Anti-CD63 recognizes an extracellular fragment of CD63, a 56 kiloDalton (kDa), type III lysosomal glycoprotein, belonging to the tetraspanin family. CD63 is expressed by granulocytes, platelets, T-cells, monocytes/macrophages and endothelial cells. CD63 protein is a canonical exosome marker and currently used to characterize exosome populations from a variety of body fluids.
Cat Num/Amount	HBM-CD63-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP, IHC



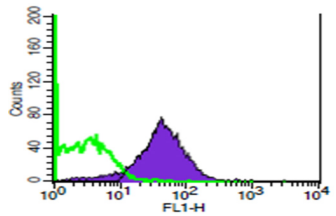
FACS
CD63 staining of exosomes purified from human plasma



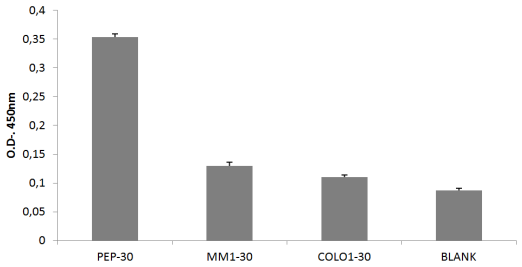
ELISA
CD63 detection in purified exosomes derived from cell supernatants (COLO1-30, MM1-30, BLCL21-30)

Anti human CD41 antibody

Description	Integrin alpha chain 2b, also known as CD41, is an heterodimeric integral membrane protein. It undergoes post-translational modifications that result in two polypeptide chains linked by a disulfide bond. CD41 is expressed on platelets and megakaryocytes, but also on early embryonic hemato-poietic stem cells and related exosomes.
Cat Num/Amount	HBM-CD41-EM1-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	FACS, ELISA



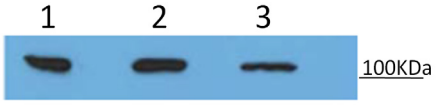
FACS
CD41 staining of exosomes purified from human plasma



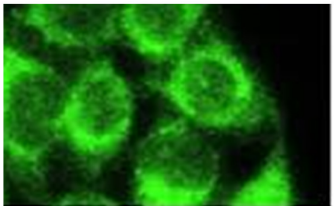
ELISA
PEP-30: 30 ug of purified exosomes from human healthy donors plasma.
MM1-30: 30 ug of purified exosomes from MM1 cell supernatant
COLO1-30: : 30 ug of purified exosomes from COLO1 cell supernatant

Anti human Alix antibody

Description	Alix protein, named also ALG2 interacting protein or PDCD6-interacting protein, is a cytoplasmic protein that interacts with apoptosis associated-proteins. Alix plays an active role in exosome bio-genesis and it is a useful internal marker for the analysis of exosomal proteins with western blot-ting.
Cat Num/Amount	HBM-ALIX-SM1-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, FACS, IF



Western blotting
1- MM1 (melanoma cell) lysate (20 ug)
2- MM1 cells purified exosomes (20 ug)
3-Plasma healthy donors purified exosomes (20 ug)

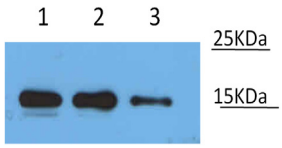


Immunofluorescence
Immunofluorescence staining of HeLA cells.

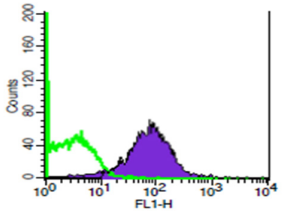
Antibodies for EV research

Anti human CD81 antibody

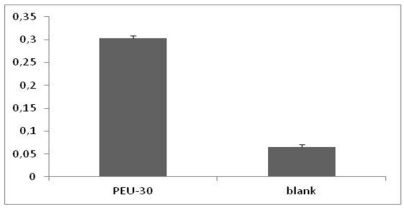
Description	CD81 (TAPA1), a member of the tetraspanin family, is virtually expressed on all nucleated cells, but in particular on germinal center B cells. CD81 forms complexes with other tetraspanin proteins, integrins and coreceptors. CD81 is expressed on exosome membranes. HBM anti-CD81 monoclonal antibody is adapt for specific antigen recognition from cell lysates and exosomes using the techniques indicated below.
Cat Num/Amount	HBM-CD81-EM1-100 (100 µg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP, IHC
References	- Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



Western blotting
1- MM1 (melanoma cell) lysate (20 ug)
2- MM1 cells purified exosomes (20 ug)
3-Plasma healthy donors purified exo-



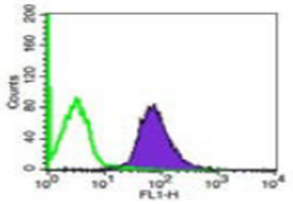
FACS
CD81 staining of COLO1 cell purified exosomes



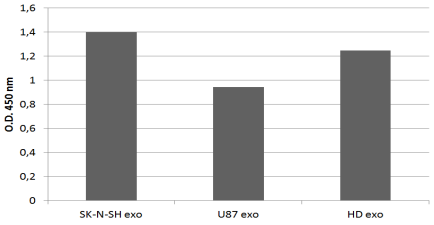
ELISA
CD81 on purified exosomes from urine (PEU), 30 ug

Anti human Flotillin antibody

Description	Flotillin belongs to the band 7.2/stomatin protein family and appears to be strongly expressed in muscle cells and fibroblasts. Flotillin expression is also correlated with Alzheimer development. Flotillin is highly expressed on exosomes and appears to be involved in exosome release mechanism. It is considered a common marker for exosomes analyses.
Cat Num/Amount	HBM-FLOT-SR1-100 (100 µg)
Type	Rabbit polyclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS
References	Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



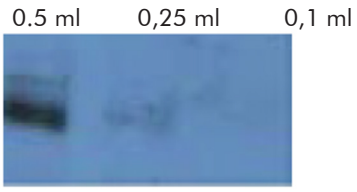
FACS
Purified exosomes from MM1 (30 ug) stained by anti-Flotillin antibody



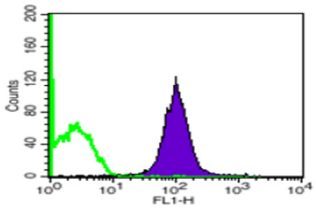
ELISA
Detection of different exosomes purified from cell culture supernatants or human plasma (HD), performed with anti-Flotillin

Anti human Rab5 antibody

Description	Rab5 is a small GTPase belonging to Ras superfamily of monomeric G proteins. Rab GTPases play an essential role in the regulation of membrane traffic and are involved in vesicle formation and transport and fusion to the membrane. Rab5 is expressed on exosome membranes and it might have an active role during endo/exocytotic processes of microvesiscles through the plasma membrane.
Cat Num/Amount	HBM-RAB5-PR1-100 (100 μg)
Type	Rabbit polyclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP
References	Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



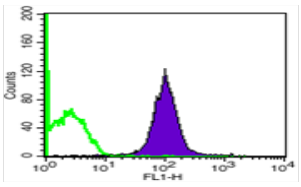
Western blotting
Exosomes purified from 0.5, 0.25, 0.1 ml of human plasma demonstrate different intensity of Rab5 expression



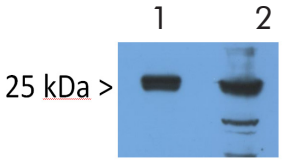
FACS
Purified exosomes from COLO1 cell line detected by Rab5 antibody

Anti mouse CD9 antibody

Description	Anti-CD9 recognizes a human 24-kiloDalton (kDa) single-chain cell-surface glycoprotein (p24) belonging to the tetraspanin family. CD9 has a very broad tissue distribution and is abundant on exosome membranes. HBM offers a monoclonal anti-CD9 antibody unconjugated reactive against mouse antigen and able to identify CD9 in mouse derived exosomes.
Cat Num/Amount	HBM-CD9M-050 (50 μg) HBM-CD9M-100 (100 μg)
Type	Mouse monoclonal unconjugated
Reactivity	Mouse
Applications	WB, ELISA, FACS, IP, IHC



FACS
CD9 staining of B16F10 mouse cell purified exosomes

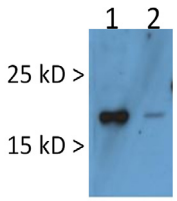


Western blotting
1- 20ug of whole lysate of B16F10 cell supernatant purified exosomes
2- 20 ug of exosomes isolated from mouse plasma

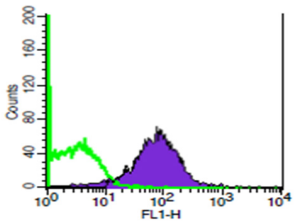
Antibodies for EV research

Anti human Caveolin antibody

Description	The lipid raft-associated protein caveolin-1 (CAV1) is the major component of the inner surface of caveolae, small invaginations of the plasma membrane. Caveolin is a transmembrane adaptor molecule that can simultaneously recognize GPI-linked proteins and interact with downstream cytoplasmic signaling molecules. It is highly expressed on exosomes derived from tumor tissue.
Cat Num/Amount	HBM-CAV1-D4-100 (100 μg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, FACS



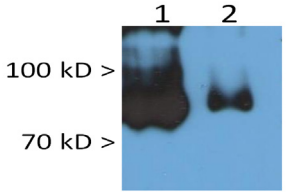
Western blotting
1-20 ug of whole lysate of melanoma cell derived exosomes (MM1)
2- 20 ug of whole MM1 cell lysate



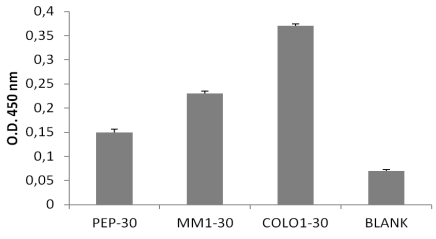
FACS
Staining of Cav1 in MM1 purified EVs

Anti human CD44 antibody

Description	CD44, known also as HCAM, is a 742 aminoacid protein involved in lymphocyte activation, homing and hematopoiesis, CD44 is expressed in multiple isoforms. CD44 is highly expressed in cancer tissues and tumor-derived exosomes, suggesting a role in tumor progression and metastasis.
Cat Num/Amount	HBM-CD44-EM1-100 (100 μg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, IP



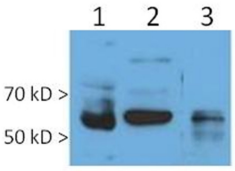
Western blotting
1- 20ug of whole lysate of MM1 cell supernatant purified exosomes
2- 20 ug of purified exosomes from human plasma of healthy donors (PEP)



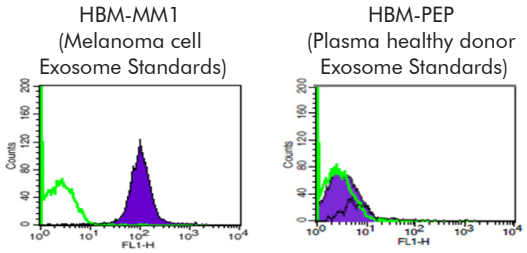
ELISA
PEP-30: 30 ug of purified exosomes from human healthy donors plasma.
MM1-30: 30 ug of purified exosomes from MM1 cell supernatant
COLO1-30: : 30 ug of purified exosomes from COLO1 cell supernatant

Anti human TM9SF4 antibody

Description	TM9SF4 (TUCAP1) is a newly discovered tumor-associated protein of unknown function that belongs to the Trans-Membrane 9 Superfamily (TM9SF). These proteins are characterized by the presence of a large variable extracellular N-terminal domain followed by nine putative transmembrane domains and a conserved C-terminal domain. TM9SF4 is mainly expressed on exosomes derived from tumor tissue. HBM offers three different monoclonal antibodies that recognize the protein on both exosomes and cell lysates.
Cat Num/Amount	HBM-SF4-PMG1-100 (100 μg)
Type	Mouse monoclonal unconjugated
Reactivity	Human
Applications	WB, FACS, IP



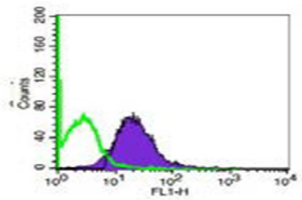
Western blotting
1- 20 ug of whole lysate of melanoma cell line
2- 20ug of whole lysate of MM1 cell supernatant purified exosomes
3- 20 ug of purified exosomes from human plasma of healthy donors (PEP)



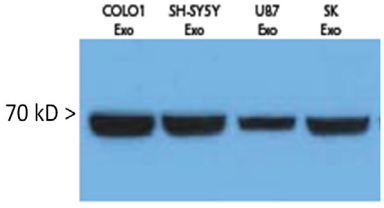
FACS
Staining of TM9SF4 on MM1 purified exosomes vs human plasma exosomes (PEP).

Anti human HSP70 antibody

Description	Heat shock protein 70 (HSP70) is a molecular chaperone that facilitates the assembly of multi-protein complexes and trafficking of polypeptides across cell membranes. HSP70 is active in promoting tumorigenesis and functions as an anti-apoptotic factor. It is highly expressed on exosomes derived from tumor tissues.
Cat Num/Amount	HBM-HSP70-SR1-100 (100 μg)
Type	Rabbit polyclonal unconjugated
Reactivity	Human
Applications	WB, ELISA, FACS, IP
References	Gagni, Paola, et al. "Combined mass quantitation and phenotyping of intact extracellular vesicles by a microarray platform." Analytica Chimica Acta (2015).



FACS
Staining of COLO1 derived exosomes with anti-HSP70

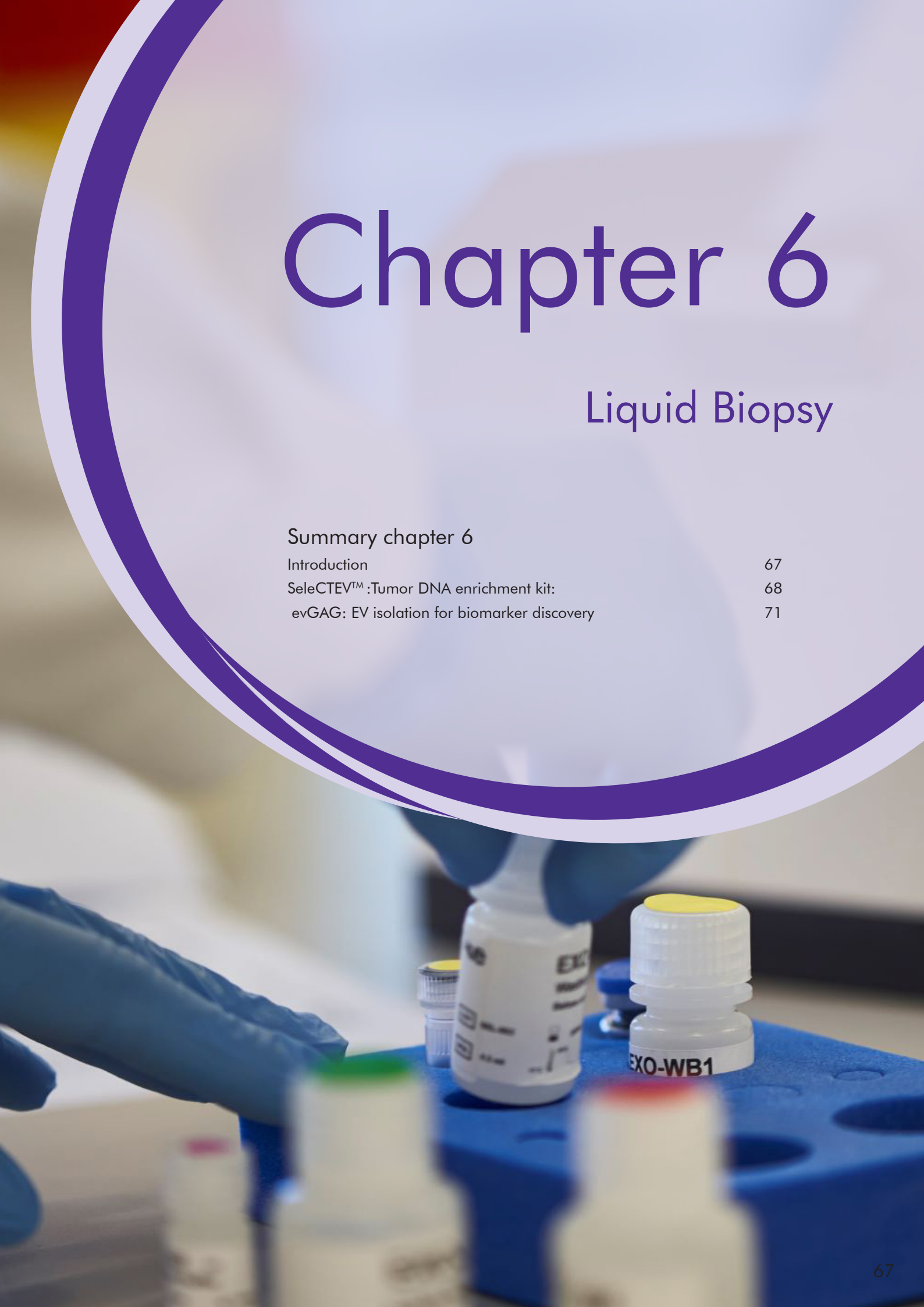


Western blotting
Analysis of HSP70 expression in different exosomes derived from tumoral cell lines:
1- COLO1; 2- SH-SYSY; 3 - U87; 4 - SK

Chapter 6

Liquid Biopsy

Summary chapter 6

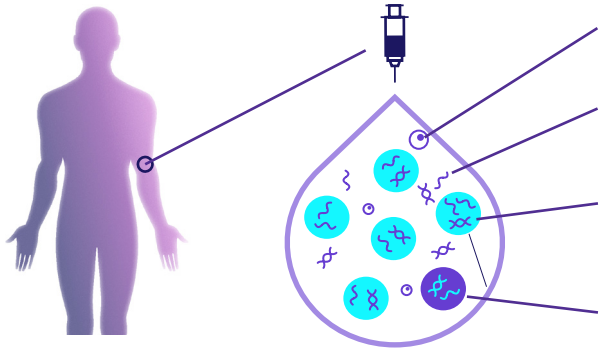


Liquid biopsy

Liquid Biopsy: introduction

Tissue biopsies are invasive and non-feasible for longitudinal monitoring of patients; moreover they do not capture the heterogeneity of the tissue or a disease of interest. Liquid biopsies are non-invasive and enable real time snapshot of the tissue homeostasis or alterations by detection of relevant biomarkers in the bloodstream. Tissue or disease specific exosomes and extracellular vesicles (EVs) can be isolated and analyzed from routine blood or urine samples featuring the ideal platform for biomarker discovery and clinical diagnostic development.

THE CHALLENGE: The major challenge and opportunity in using EVs based liquid biopsy lies in the tremendous complexity of biofluid samples, heterogeneity of exosomes and extracellular vesicles and the low abundance of specific tissue or disease markers.



CIRCULATING TUMOR CELLS (CTCSs).
Cancers may shed cells into the bloodstream. Very rare.

CIRCULATING FREE NUCLEIC ACIDS (cfRNA, cfDNA).
Mostly coming from dead cells. cfRNA is usually degraded.

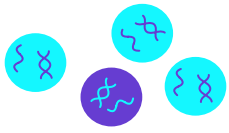
EXOSOMES AND EVs
Shuttling in the body fluids markers of parental cells.

TUMOR-DERIVED EXOSOMES AND EVs
Secreated by tumor cells in the bloodstream and body fluids.

THE SOLUTION: HansaBioMed Life Sciences provides a range of pre-analytical solutions, empowered by Exosomics s.p.a, to isolate either overall EVs or selectively enrich for specific subpopulations (i.e. tumor derived-EVs) from biofluids with high yield and purity, and further extract their DNA and RNA content. Our technologies are efficient in both biomarker discovery and confirmation studies, and comply with clinical grade analytical platforms. In clinical settings, our solutions enable ultrasensitive detection of tumor associated mutations and RNA molecules, enabling next generation of tumor screening, monitoring, staging and monitoring tests.

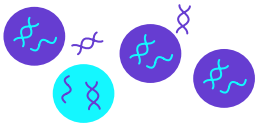
Pre-analytical solutions

Isolation of total EVs for biofluids for biomarker discovery



EV-GAG: fast isolation of EVs form biofluids

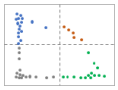
Enrichment of Tumour derived EVs and nucleic acid isolation



SeleCTEV™: Tumor DNA enrchment kit

Analytical services

Mutation analysis and biomarker screening



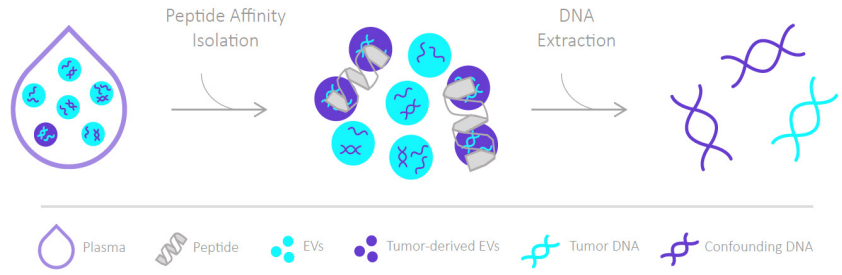
Digital PCR

Real time qPCR

NGS

SeleCTEV™ :Tumor DNA enrichment kit

SeleCTEV™ DNA Enrichment Kit allows the selective purification of circulating free DNA (cfDNA) and tumor-derived extracellular vesicles (EVs) DNA from plasma. The isolation is based on Exosomics' proprietary peptide affinity method.



Cat. Code	Description	Size
SeleCTEV: Tumor DNA enrichment kit		
HBM-EXS-DNA	Tumor DNA enrichment kit	24 reactions

Applications

- Pre-analytical purification of tumor-derived EVs. and circulating DNA (cfDNA).
- Isolated DNA suitable for the detection of actionable mutations by digital PCR (dPCR).

Characteristics

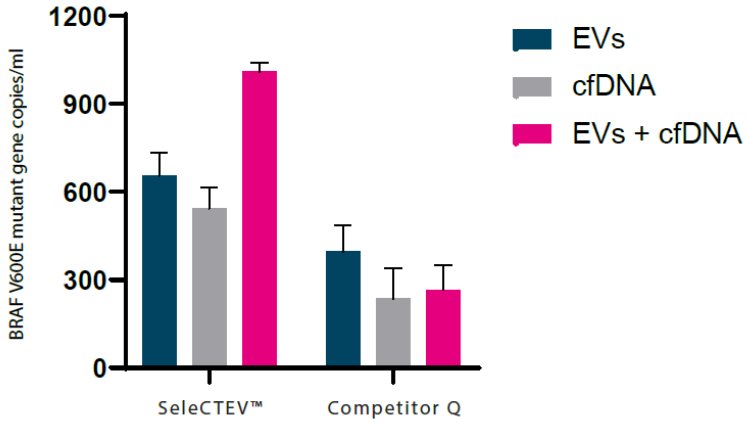
- Peptide affinity EV and DNA pull down.
- Sample type: human plasma, serum, urine.
- Sample volume: 0.5 - 2 ml of fluid.

Advantages

- Efficient enrichment of tumor-derived EVs.
- Combines EV capture and genomic DNA isolation.
- Suitable for downstream dPCR, qPCR, NGS.

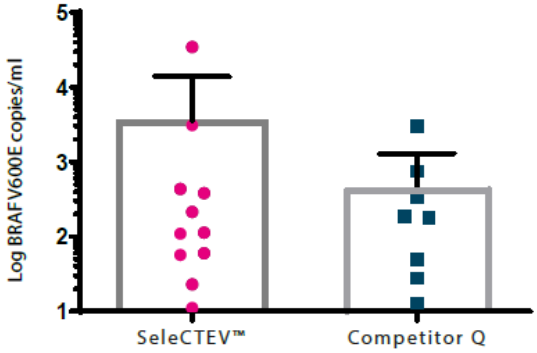
SeleCTEV™: Tumor DNA enrichment kit. Mutation recovery

Mutation-bearing EVs and cfDNA were spiked alone and together into healthy donor plasma and processed with SeleCTEV™ and Competitor Q to obtain DNA. SeleCTEV™ isolated more mutation than Competitor Q from both biological sources, suggesting that SeleCTEV™ is a more efficient way to isolate EVs and cfDNA than Competitor Q.

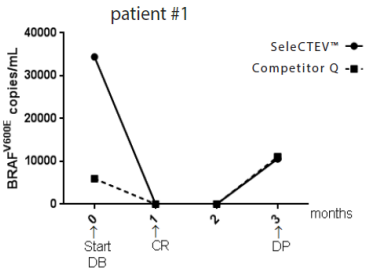


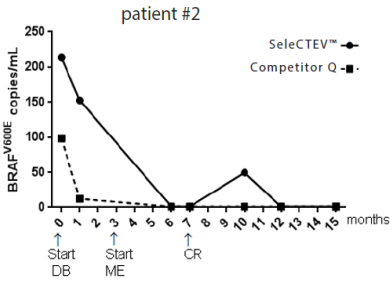
SeleCTEV™: Tumor DNA enrichment kit. Case study, metastatic melanoma patients

Plasma samples were collected from twenty patiens with BRAF V600E positive tumors and thirty patients with wild type (WT) metastatic melanoma (MM) based on tissue biopsy examination. Copies of BRAV V600E and BRAF WT were detected by digtal PCR. BRAF V600E gene copies were detected in 11 - and 8 Competitor Q - processed plasma samples of the mutant cohort.



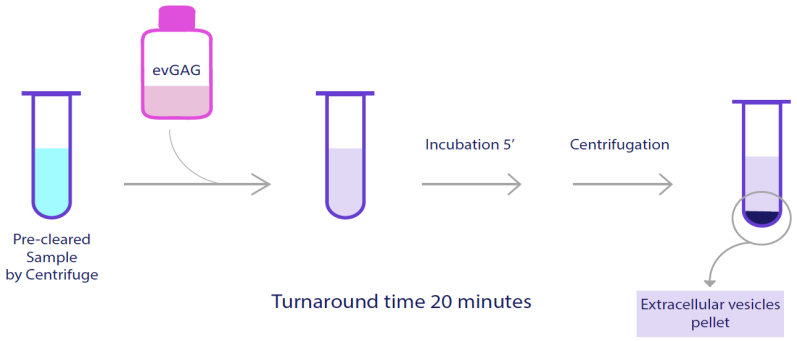
SeleCTEV™ and Competitor Q were used for monitoring BRAFV600E levels in the plasma of BRAF inhibitor-treated MM patients.
In patient #1 disease progression (DP) occurred within 3 months and was associated to rebounding levels of circulating BRAFV600E and unfavorable prognosis. In patient #2 no clinical evidence of disease progression was observed at later time points, and mutant gene copies remained low or undetectable in plasma.





evGAG: EV isolation for biomarker discovery

evGAG is a patented isolation method that allows precipitation of extracellular vesicles (EVs) from biofluids. The evGAG reaction is based on the interaction between the precipation solution and glycosaminoglycans (GAGs) in the EVs.The product is ideal for the discovery of EV associated biomarkers.



Cat. Code	Description	Size
SoRTEV: Tumor RNA enrichment kit		
HBM-EXS-GAG	Tumor RNA enrichment kit	24 reactions

Applications

- Isolation of EVs from biofluids.
- Discovery of EV associated biomarkers.
- Efficient for EVs isolation from urine

Characteristics

- Affinity isolation method.
- Sample type: Urine and diluted biofluids.
- Sample volume: 0.5 - 2 ml

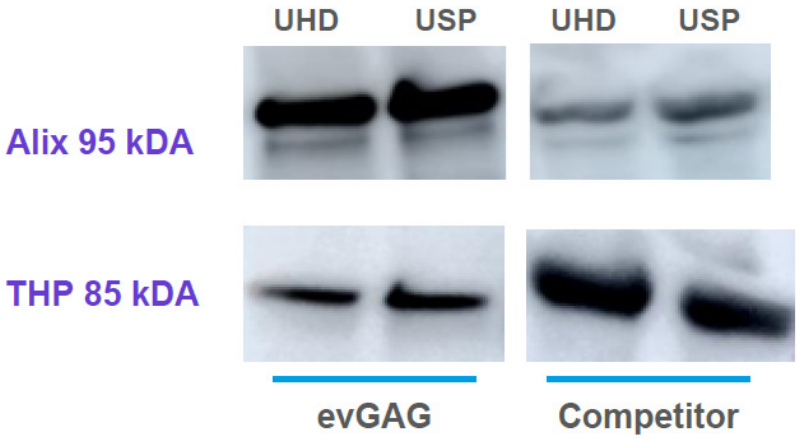
Advantages

- Rapid turnaround time (20 min).
- Small sample volume required.
- Simple procedure and high yield recovery.

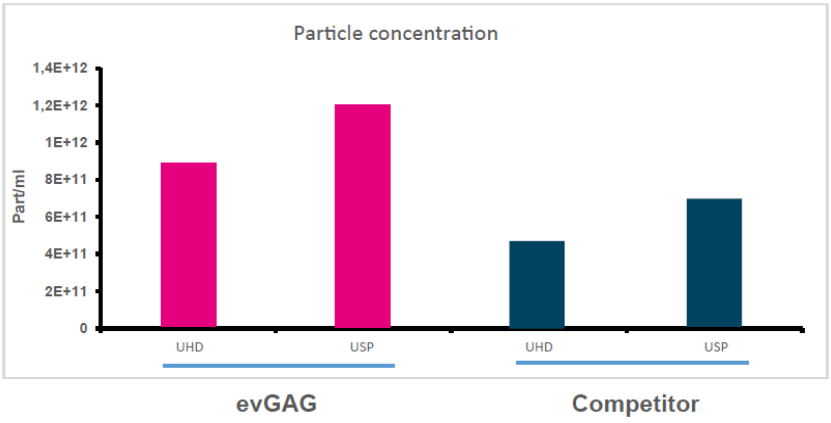
Performance of evGAG technology in isolating EVs from urine samples

Urine samples were processed with evGAG. Briefly, 1 mL of urine from healthy donor (UHD) and 1 mL of urine from healthy donor spiked-in (USP) with extracellular vesicles purified from colon cancer cell line containing KRASG13D mutation (cat nr. EXO-REF-KRAS-G13D-2) were incubated with 2 mL of evGAG each, for 5 minutes and then centrifuged at 3,000g for 15 minutes. This results in a precipitated pellet containing EVs.

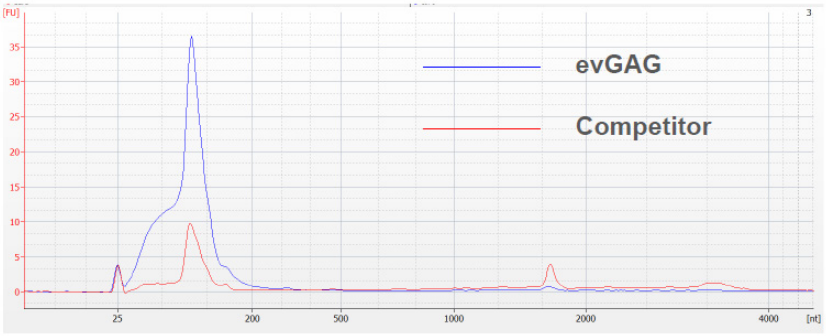
Western Blot Analysis of extracellular vesicle markers Alix, confirmed that the concentration of EVs isolated by evGAG was higher compared to the competitor. On the contrary, uromoduline (THP) as not specific target is less abundant in EVs isolated by evGAG than competitor.



The pellet containing EVs was re-suspended in PBS and analyzed by Nanoparticle Tracking Analysis (NTA). Nanoparticles concentration is 2 times higher in EVs isolated with evGAG in both UHD and USP samples (8.8 x 10^{11} and 1.2 x 10^{12} respectively) than to EVs isolated with competitor in both UHD and USP samples (4.6 x 10^{11} and 6.9 x 10^{11} respectively).



EV RNA was isolated from urine using the extraction phase of SoRTEV™ and analyzed with Bioanalyzer. The results showed that EVs purified with evGAG contain the highest yield of small RNA content (<200 nt) with a main peak at 100 nt indicating an efficient extraction of small exoRNA from urine, opposed to EVs isolated with competitor kit.



Chapter 7

Service and Collaborations

Summary chapter 7

Services and collaborations

Experience: Being the oldest company entirely dedicated to Extracellular Vesicle research since 2007, Our scientists have over 10 years of experience in EV area, high background in molecular and cell biology, in cancer research, and development of diagnostics.

Custom design: We will custom design your solution to meet your specific requirements.We are part of an international network of key opinion leaders, life science and biotech experts.

High project management skills: Ensuring a successful outcome begins and ends with managing our client's projects. We have a deep expertise in project management, participating in the framework of international projects together with Academic and Industrial partners.

Extracellular Vesicle isolation and purification

HansaBioMed Life Sciences provides high expertise in Extracellular Vesicles purification from human or animal biofluids, cell conditioned media, microbial cultures and plants. EV purification can be obtained following our standard protocol which combine tangential flow filtration and SEC. Isolated EVs are subsequently quantified for total protein contents and validated by nano tracking analysis (NTA) with the Zetaview equipment (Particle Metrix). Purified EVs can be shipped to the customer lyophilized or frozen, as required.

Extracellular Vesicle isolation and purification			
Service	Material	Standard method	Alternative methods
Small and Large EVs purification	Cell conditioned media, including MSC.	Tangential fow filtration and size exclusion chromatography	Ultracentrifugation Immunoaffinity Chemical precipitation
	Human or animal biofluids (plasma, serum, urine, CSF, saliva, milk)		
	Plants (fruits and vegetables)		
	Microbe cultures (bacteria, parasites)		

Extracellular Vesicle characterization and phenotyping

Services for characterization of the Extracellular vesicles, analysis of particle size distribution and concentration, OMICS analyses, biomarker discovery.

Extracellular Vesicle characterization and analysis		
Service	Material	Method available
Particle size distribution, concentration, zeta potential.	Purified EVs	Nanoparticle tracking analysis (NTA) in scattered or fluorescence mode (Zetaview, Particle Metrix). DLS analysis.
Flow cytometry	Purified EVs	Analysis by NanoFCM analyzer.
Transmission Electron Microscopy (TEM)	Purified EVs	EV image, immunoEM.
EV phenotyping	Purified EVs, raw material	ELISA high throughput marker screening

Extracellular Vesicle OMICS		
Service	Material	Method available
Mass spectrometry	Purified EVs	Sample preparation and Q Exactive Plus 2h nano-LC/MS/MS analysis with MaxQuant-based identification and quantitation.
miRNA sequencing	Purified EVs	Library preparation with Nextflex kit. Sequencing done by Illumina Nextseq 500

Custom products

HansaBioMed Life Sciences can design and create a personalized kit by choosing from a wide variety of reagents and tools in our catalog.

Custom products	
Product	Description
Custom ELISA immunoplates	ELISA plates can be covalently coated with EV-binding antibodies. 1- Trasparent, white and black plates are available. 2- Coating antibody can be chosen by our antibody list (see section 6) or sent to HBM by customers.
Custom immunobeads	Latex immunobeads will be covalently coupled with the antibodies chosen from our antibody list, or sent to HBM-LS by customers. We provide the following product sizes: 1- Immunobeads for 10 reactions 2- Immunobeads for 20 reactions 3- Immunobeads for 50 reactions
Custom ExoTEST	Customer can customize our ExoTEST platform chosing ELISA plate and detection antibody (see section 3, Quantification kits)
For other Custom made solutions and for information contact us at info@hansabiomed.eu or visit our website	

Other services and project collaboration

HansaBioMed Life Sciences is open for collaboration with private or academic partners in research activites related to different fields of exosome research and applications. Contact us for:

Service and project collaboration	
Service	Description
Biomolecule labeling and modification	We can provide biomolecule modifications such as: protein labeling with fluorescence dyes, conjugation of biomolecules with affinity ligands, PEGyation of biomolecules, immobilization of biomolecules on different surfaces (hydrogels, molded polymers, synthetic membranes) etc.
New assays for marker discovery	We can develop personalized ELISA assays for a specific exosome protein marker quantification or qPCR assays for identification of miRNA and DNA markers.
Development of tools for capturing and enriching specific exosome subpopulations	We can develop tools for capturing and isolating specific exosome subpopulations to improve comprehensive exosome profiling in diseases.
Projects and collaborations and technical consulting	Contact us for: OEM product development, cell free regenerative medicine, collaboration in research projects (Horizon 2020, Eurostars, NIH grants, etc).

Sambo Medical Co.

Distribution of HansaBioMed Life Sciences products in South Korea

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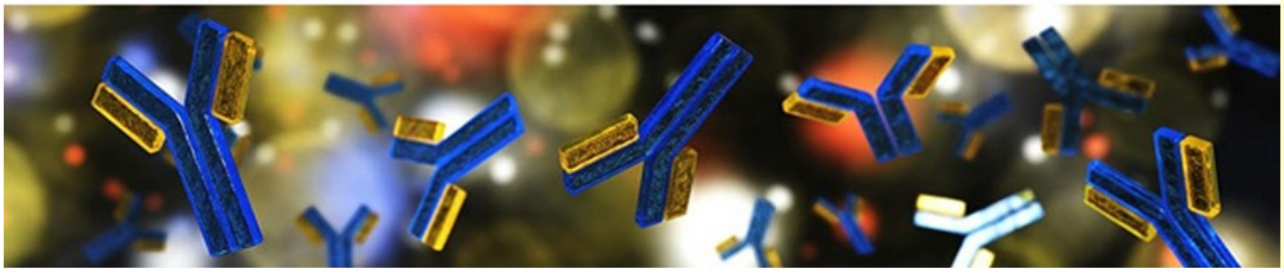

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15 Years of Extracellualar Vesicles experience

HansaBioMed Life Sciences

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