**ExoCap™ for Serum Plasma**  
**Exosome Isolation and Enrichment Kits**

**ExoCap™ CD9 Kit for Serum Plasma**  
**ExoCap™ CD63 Kit for Serum Plasma**  
**ExoCap™ CD81 Kit for Serum Plasma**  
**ExoCap™ EpCAM Kit for Serum Plasma**  
**ExoCap™ Composite Kit for Serum Plasma**

**PRODUCT DESCRIPTION**

ExoCap™ for Serum Plasma is a kit for exosome isolation and enrichment designed and optimized for use with serum, plasma and cell culture supernatant. Exosomes are extracellular vesicles secreted by most cell types and contain these marker proteins and microRNAs. This kit consists of: 3 µm magnetic Capture Beads coupled with antibodies that recognize exosome surface antigens CD9, CD63, CD81 or EpCAM; Treatment Buffer that decreases the non-specific binding on the magnetic bead; Washing/Dilution Buffer and Exosome Elution Buffer.  
ExoCap™ uses functionalized Magnosphere™ magnetic microparticles (Capture Beads) for exosome separation. These beads are coated with a JSR Life Sciences proprietary hydrophilic polymer to decrease non-specific binding and antibody which specifically bind to exosomes.

**FEATURES**

- Specific capture of exosomes from serum, plasma, and cell culture supernatant.  
- Immunoprecipitation method using magnetic beads enables automation.  
- Five kits acceptable according to exosome target.  
- Simple and flexible protocol according to your target abundance.  
- All components are BSA free.  
- Isolation of exosomes from ExoCap™ beads.  
- Treatment Buffer designed for high-protein samples such as serum and plasma.

**EXAMPLE APPLICATIONS**

ExoCap™ enables you to separate exosomes from serum, plasma and cell culture supernatant. Isolated exosomes can be used for western blot, ELISA, electron microscopy, flow cytometry and qRT-PCR.
PRODUCT COMPONENTS

<table>
<thead>
<tr>
<th>ExoCap™ Components</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Each Capture Beads*</td>
<td>2 mL</td>
</tr>
<tr>
<td>Treatment Buffer</td>
<td>30 mL</td>
</tr>
<tr>
<td>Washing/Dilution Buffer</td>
<td>60 mL</td>
</tr>
<tr>
<td>Exosome Elution Buffer</td>
<td>1.5 mL</td>
</tr>
</tbody>
</table>

TEST NUMBERS BY DOWNSTREAM APPLICATION

<table>
<thead>
<tr>
<th>Downstream Application</th>
<th>Test numbers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western blotting</td>
<td>20 tests</td>
</tr>
<tr>
<td>Flow cytometer analysis</td>
<td>40 tests</td>
</tr>
<tr>
<td>Nucleic acid detection (microRNA)</td>
<td>4 tests</td>
</tr>
</tbody>
</table>

*Test number is our recommendation. Customer may titrate beads amount according to sample target abundance.

EACH KIT CONTAINS THESE CAPTURE BEADS

<table>
<thead>
<tr>
<th>ExoCap™</th>
<th>Capture Beads</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD9 Kit</td>
<td>CD9 Capture Beads</td>
<td>Beads diameter: 3 μm</td>
</tr>
<tr>
<td>CD63 Kit</td>
<td>CD63 Capture Beads</td>
<td></td>
</tr>
<tr>
<td>CD81 Kit</td>
<td>CD81 Capture Beads</td>
<td></td>
</tr>
<tr>
<td>EpCAM Kit</td>
<td>EpCAM Capture Beads</td>
<td>Solid content: 0.2%</td>
</tr>
<tr>
<td>Composite Kit</td>
<td>Mixture of CD9, CD63, CD81, EpCAM Capture Beads</td>
<td>(1.2 × 10⁸ beads/mL approx.)</td>
</tr>
</tbody>
</table>

STORAGE
Store at 2-8°C, DO NOT FREEZE.

REQUIRED EQUIPMENT
- Magnetic tube stand.
- Vortexer and sample shaker.
- Buffer for suspending bead-exosome complex.
- Gel filtration device or ultrafiltration for buffer exchange [optional].

RECOMMENDED PROTOCOLS FOR CAPTURING EXOSOME

PREPARATION SHEET

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment Buffer†</th>
<th>Capture Beads† †</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μL</td>
<td>100 μL</td>
<td>100 μL (Western blotting)</td>
<td>200 μL</td>
</tr>
<tr>
<td>250 μL</td>
<td>250 μL</td>
<td>50 μL (Flow cytometer)</td>
<td>500 μL</td>
</tr>
<tr>
<td>500 μL</td>
<td>500 μL</td>
<td>500 μL (qPCR for microRNA)</td>
<td>1000 μL</td>
</tr>
<tr>
<td>1000 μL</td>
<td>1000 μL</td>
<td>(Remove dispersion liquid before use)</td>
<td>2000 μL</td>
</tr>
</tbody>
</table>

†For low-protein sample such as cell culture supernatant, you may substitute Treatment Buffer with Washing/Dilution Buffer as needed.
Standard usage. Customer can titrate the amount of Capture Beads according to target abundance and application.

Protocol 1. [Cell and Debris Pre-Clearance Procedure]
1. Prepare the appropriate size tubes for your sample.
2. Dispense the sample into tube.
3. Centrifuge the tubes at 300 x g at 4°C for 10 minutes.
4. Transfer supernatant to a new tube.
5. Centrifuge the tubes at 2000 x g at 4°C for 20 minutes.
6. Transfer supernatant to a new tube.
7. Centrifuge the tubes at 10,000 x g at 4°C for 30 minutes.
8. (Optional) Filter the final supernatant with a 0.22 μm filter unit.
9. The sample is now ready for immediate use with ExoCap™ kit or storage at -80°C.

Protocol 2. [Exosome Capture Procedure for Western blotting]
Example: 100 μL* beads, 1 mL sample
*Recommended beads amount for Western blotting sample preparation. You may titrate the Capture Beads amount according to your target abundance.
1. Set 2 mL microfuge tubes on a magnetic tube stand.
2. Suspend ExoCap™ Capture Beads well using a vortexer and put 100 μL of the suspension into each microfuge tubes per sample.
3. Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
4. Add 1000 μL of Treatment Buffer for serum and plasma, or Washing/Dilution Buffer for cell culture supernatant.
5. Add 1000 μL of sample that has been cleared of cells and debris. (Note1: please see above protocol 1. Cell and Debris Pre-Clearance Procedure, Note2: Customer may use as little as 100 μL sample from according to target abundance.)
6. Incubate the sample for 20 minutes - 24 hours at room temperature or 4°C with gentle mixing. (Note: the optimal reaction time and temperature may depend on the target abundance and stability.)
7. Briefly spin the tube to remove beads from the top of the tube.
8. Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
9. Wash the beads 2 times with 500 μL Washing/Dilution Buffer. Mix the beads briefly but thoroughly. After 2 times washing, resuspend with 500 μL Washing/Dilution Buffer and transfer whole this buffer containing ExoCap™ Capture Beads to a fresh tube.
10. Place the tube on the magnetic tube stand for about a minute and remove the supernatant carefully.

Move to either ‘Preparation Protocol for SDS-PAGE’ or ‘Exosome Elution Procedure’.

Option) In order to elute exosome proteins for Western blotting, you may add the appropriate lysis buffer directly to the beads according to your preferred method/kit. Place the tube on the magnetic tube stand for about a minute and collect the eluted sample.
Protocol 3. [Preparation Protocol for SDS-PAGE]
Continued from protocol 2,
1. Add 20 µL of 1x Sample Buffer to the beads and mix well.
2. Incubate 95°C for 5 minutes.
3. Vortex and spin down.
4. Place the tube on magnetic tube stand about a minute.
5. Apply the total supernatant to a lane of SDS-PAGE.

Protocol 4. [Exosome Elution Procedure]
Continued from protocol 2,
1. Add 25-50 µL of Exosome Elution Buffer to resuspend the beads. Mix gently by pipetting (do not use a vortexer).
2. Incubate the beads without mixing for 3 minutes at room temperature.
3. Place the tube on the magnetic tube stand for about a minute and transfer the supernatant to a fresh tube.
4. Immediately proceed to exchange the Exosome Elution Buffer to Washing/Dilution Buffer or your preferred buffer, such as PBS etc. Products such as Amicon Ultra-0.5mL Centrifugal Filters (100 kDa) or GE Healthcare illustra MicroSpin G-25 Columns can be used. Please refer to supplier’s protocol for detailed instructions.

Note: Exosome Elution Buffer contains a denaturing agent. The Exosome Elution Buffer may interfere with downstream applications unless diluted to 20-100 times or exchanged with Washing/Dilution Buffer or your preferred buffer. Washing/Dilution Buffer contains a synthetic polymer to reduce adsorption of exosomes on tubes and buffer exchange devices. However, the polymer may disturb certain downstream analysis of the exosomes.

Protocol 5. [Exosome Capture Procedure for flow cytometry]
Example: 50 µL* beads, 300 µL sample
*Recommended Capture Beads amount for flow cytometry sample preparation. You may titrate the magnetic beads amount according to your target abundance.
1. Set 2 mL microfuge tubes on a magnetic tube stand.
2. Suspend ExoCap™ Capture Beads well using a vortexer and put 50 µL of the suspension into each microfuge tubes per sample.
3. Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
4. Add 300 µL of Treatment Buffer for serum and plasma, or Washing/Dilution buffer for cell culture supernatant.
5. Add 300 µL of sample that has been cleared of cells and debris. (Note 1: please see above protocol 1. Cell and Debris Pre-Clearance Procedure, Note 2: Customer may use as little as 100 µL sample from according to target abundance.)
6. Incubate the sample for 24 hours at room temperature or 4°C with gentle mixing. (Note: the optimal reaction time and temperature vary with the target abundance.)
7. Briefly spin the tube to remove beads from the top of the tube.
8. Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
9. Wash the beads 2 times with 250 µL Washing/Dilution Buffer and resuspend in 300 µL PBS or your preferred buffer.
Protocol 6. [Preparation of exosome-bead complexes for flow cytometer analysis]
Continued from protocol 5,
1. Add optimized volume of fluorescent antibody of a target or isotype control to 100 μL of prepared exosome-beads complex. Mix gently by rotator for 1 hour. (Note 1: titration of antibody conditions are important for optimal signal, Note 2: A variety of Isotype control fluorescent antibodies might be available from MBL (MEDICAL & BIOLOGICAL LABORATORIES. CO., LTD.)
2. Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
3. Wash the beads 1 time with 300 μL PBS or your preferred buffer.
4. Add 300 μL of PBS or your preferred buffer for flow cytometry.
5. Perform flow cytometer analysis.

Protocol 7. [Exosome Capture Procedure for quantitative PCR]
Example: 500 μL* beads, 1 mL sample
*Recommended Capture Beads amount for qPCR sample preparation. You may titrate the magnetic beads amount according to your target abundance. We recommend 1 mL sample volume. Samples less than 1 mL may deteriorate Ct value.
1. Set 2 mL microfuge tubes on a magnetic tube stand.
2. Suspend ExoCap™ Capture Beads well using a vortexer and put 500 μL of the suspension into each microfuge tube per sample.
3. Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
4. Add 1000 μL of Treatment Buffer for serum and plasma, or Washing/Dilution Buffer for cell culture supernatant.
5. Add 1000 μL of sample that has been cleared of cells and debris. (Note 1: please see above protocol 1. Cell and Debris Pre-Clearance Procedure, Note 2: Customer may use as little as 100 μL sample from according to target abundance.)
6. Incubate the sample for 24 hours at room temperature with gentle mixing. (Note: the optimal reaction time and temperature vary with the target abundance.)
7. Briefly spin the tube to remove beads from the top of the tube.
8. Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
9. Wash the beads 2 times with 500 μL Washing/Dilution Buffer. Mix the beads briefly but thoroughly. Re-suspend with 500 μL and transfer the final washed beads to a fresh tube.
10. Place the tube on the magnetic tube stand for about a minute and remove the supernatant carefully.
11. Exosomes bound with ExoCap™ Capture Beads are ready for nucleic acids isolation.

(Option) For nucleic acid isolation, ExoCap™ Nucleic Acid Elution Buffer, Code No. MEX-E is available from MBL (MEDICAL & BIOLOGICAL LABORATORIES. CO., LTD.). Obtained nucleic acids can be used as a qPCR template. For cDNA synthesis reaction or qPCR reaction, please follow supplier’s instructions and use the isolated nucleic acids as much as possible if necessary.
EXPERIMENTAL EXAMPLE

Western blotting analysis from Serum and Plasma of Healthy donor

qPCR (miR-21) analysis from Serum of Healthy donor

time and beads amount dependence

Flow cytometer analysis from Cell culture supernatant

time and sample volume dependence
IMPORTANT NOTICE AND TERMS AND CONDITIONS

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