HTRANSFECTION EXPERTS

Virus Production

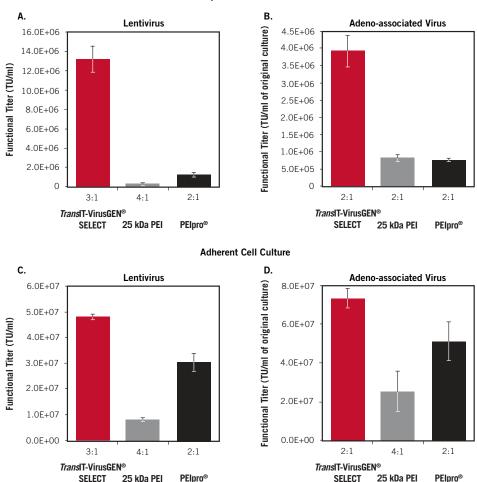
NOW Available! **150 ml size**







TransIT-VirusGEN[®] SELECT Outperforms PEI and PEIpro[®]



Suspension Cell Culture

Lentivirus was produced using (A) suspension FreeStyle[™] 293-F cells grown in FreeStyle[™] F17 Medium or (C) adherent 293T/17 cells grown in DMEM + 10% FBS and transfected with 3rd generation vectors pLK0.1-puro-CMV-TurboGFP[™] transfer vector (Sigma) and ViraSafe[™] Pantropic Packaging mix (pRSV-Rev, pCMV-VSV-G, pCgpV, Cell Bio Labs) at a 3:0.5:0.5:2 DNA ratio, 1 µg/ml total plasmid DNA, using the *Trans*IT-VirusGEN[®] SELECT Transfection Reagent (3:1, vol:wt), 25 kDa linear PEI (4:1, Polysciences) or PEIpro[®] (2:1, Polyplus Transfection). Virus-containing supernatant was used to transduce 293T/17 cells and GFP expression was measured at 72 hours post-transduction using a Guava[®] easyCyte[™] 5HT Flow Cytometer.

AAV2 was produced using (B) suspension FreeStyle[™] 293-F cells grown in FreeStyle[™] F17 Medium or (D) adherent 293T/17 cells grown in DMEM + 10% FBS and transfected using pAAV-hrGFP, pAAV-RC, and pAAV-Helper plasmids (1:1:1 DNA ratio, 1.5 µg/ml plasmid DNA, Agilent Technologies) using *Trans*IT-VirusGEN[®] SELECT Transfection Reagent (2:1, vol:wt), 25 kDa linear PEI (2:1 or 4:1, Polysciences) or PElpro[®] (2:1, Polyplus Transfection). Harvested virus was used to transduce HT1080 cells and GFP expression was measured 48 hours post-transduction using a Guava[®] easyCyte[™] 5HT Flow Cytometer. For both lentivirus and AAV, functional titers were measured from virus dilutions with less than 20% GFP positive cells.*

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Animal Origin Free Documentation

Below is an example of the Certificate of Origin and BSE/TSE Statement for *Trans*IT-VirusGEN[®] SELECT Transfection Reagent documenting that it is chemically synthesized and it is not manufactured with any animal-derived components.

CERTIFICATE OF	ORIGIN	Mirus	I
Product Name:	TransIT-VirusGEN® SELECT TI	ransfection Reagent	
Product Number:	MIR 6730	C .	
Lot Number:			
Animal Derived Components	None		
Type of Manufacture:	Chemical Synthesis		
Country of Manufacture:	USA		
This information is to be used for "country of origin" for import/exp	the purpose of determining animal origin ort purposes.	only and not to be confused with	
BS	E TSE Statement		Mirus.
Produ Lot N	Int Name: TransIT-VirusGEN® SEL Int Number: MIR 6730 umber: Date:	ECT Transfection Reagent	
—			
packag produc Encepi Mirus or TSE	ing, storage and transportation of these mate ts have minimal risk of contamination with I halopathy (TSE).	A) entirely from material of non-animal origin. Trials do not involve the use of material of animal Bovine Spongiform Encephalopathy (BSE) or Tra- roduction of this material in any way that would	origin. Therefore, these insmissible Bovine
Susan Elder, Quality Contr			
This product is sold to the Buyer with product, may not be re-packaged or re	son Elden		
	Elder, Quality Supervisor		
Minus Bio LLC (5602	4		
	Mirus Bio LLC 5602 Research Park Blvd. Ste 210 Ma	dison, Wisconsin 53719 USA 608.441.2852 FAX 608.441.2849 wa	vw.mirusbio.com



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Quality Control Documentation

Below is an example of the Certificate of Analysis for *Trans*IT-VirusGEN[®] SELECT Transfection Reagent that includes a functional assay and testing for: sterility, endotoxin and mycoplasma.

CERTIFICATE OF ANALYSIS



Product Name: TransIT-VirusGEN® SELECT Transfection Reagent Product Number: MIR 6730 Lot Number: Date of Manufacture: Retest Date: Storage Condition: -10 to -30°C Issue Date:

Quality Control Testing and Results

Description	Specification	Result
Functional Assay ¹	> 500,000 TU/mL	Pass
Sterility Testing ²	No growth observed	Pass
Endotoxin Testing3	$\leq 1 \text{ EU/mL}$	Pass
Mycoplasma Testing ⁴	None detected	Pass
Identity	All components detected	Pass
Appearance	Clear, colorless solution	Pass

References

¹*Trans*IT-VirusGEN[®] SELECT Transfection Reagent is tested for adeno-associated virus (AAV) production in suspension 293 cells using a GFP encoding transfer vector. Functional virus titer is determined by transducing HT1080 cells and measuring GFP expression by flow cytometry.

² Performed per USP <71> guidelines.

- ³ Performed per USP <85> guidelines.
- ⁴ Performed per USP <63> guidelines.

Certified By: Susan Elden

Susan Elder, Quality Supervisor

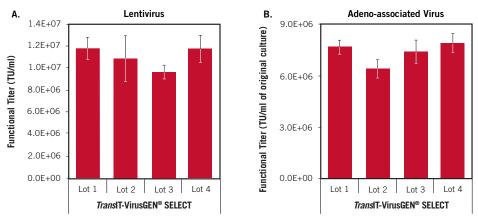
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TransIT-VirusGEN® is a registered trademark of Mirus Bio LLC.

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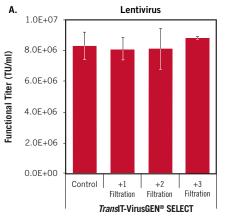
Reliable Lot-to-Lot Consistency

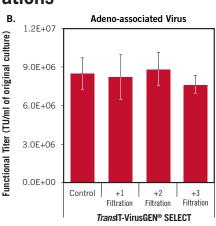


(A) Lentivirus was produced using suspension FreeStyle[™] 293-F cells grown in FreeStyle[™] F17 Medium and transfected with 3rd generation vectors pLK0.1-puro-CMV-TurboGFP[™] transfer vector (Sigma) and ViraSafe[™] Pantropic Packaging mix (pRSV-Rev, pCMV-VSV-G, pCgpV, Cell Bio Labs) at a 3:0.5:0.5:2 DNA ratio, 1 µg/ml total plasmid, using the *Trans*IT-VirusGEN[®] SELECT Transfection Reagent (3:1, vol:wt). Virus containing supernatant was used to transduce 293T/17 cells and GFP expression was measured at 72 hours post-transduction using a Guava[®] easyCyte[™] 5HT Flow Cytometer.

(B) **AAV2** was produced using suspension FreeStyle[™] 293-F cells grown in FreeStyle[™] F17 Medium and transfected using pAAV-hrGFP, pAAV-RC and pAAV-Helper plasmids (1:1:1 DNA ratio, 1.5 µg/ml, Agilent Technologies) using *Trans*IT-VirusGEN[®] SELECT Transfection Reagent (2:1, vol:wt). Harvested virus was used to transduce HT1080 cells and GFP expression was measured 48 hours post-transduction using a Guava[®] easyCyte[™] 5HT Flow Cytometer. For both lentivirus and AAV, functional titers were measured from virus dilutions with less than 20% GFP positive cells.^{*}

Compatible with Multiple Filtrations





(A) Lentivirus was produced using suspension FreeStyle[™] 293-F cells grown in FreeStyle[™] F17 Medium and transfected with 3rd generation vectors pLKO.1-puro-CMV-TurboGFP[™] transfer vector (Sigma) and ViraSafe[™] Pantropic Packaging mix (pRSV-Rev, pCMV-VSV-G, pCgpV, Cell Bio Labs) at a 3:0.5:0.5:2 DNA ratio, 1 µg/ml total plasmid, using the *Trans*IT-VirusGEN[®] SELECT Transfection Reagent (3:1, vol:wt) that was filtered through a 0.22 um polyethersulfone (PES) filter unit (Millipore Sigma) for the indicated number of times. Virus-containing supernatant was used to transduce 293T/17 cells and GFP expression was measured at 72 hours post-transduction using a Guava[®] easyCyte[™] 5HT Flow Cytometer.

(B) **AAV2** was produced using suspension FreeStyle[™] 293-F cells grown in FreeStyle[™] F17 Medium and transfected using pAAV-hrGFP, pAAV-RC and pAAV-Helper plasmids (1:1:1 DNA ratio, 1.5 μg/ml, Agilent Technologies) using *Trans*IT-VirusGEN[®] SELECT Transfection Reagent (2:1, vol:wt). Harvested virus was used to transduce HT1080 cells and GFP expression was measured 48 hours post-transduction using a Guava[®] easyCyte[™] 5HT Flow Cytometer. For both lentivirus and AAV, functional titers were measured from virus dilutions with less than 20% GFP positive cells.*

*The error bars represent the range of triplicate wells. Suspension cells were at a density of 2×10^6 cells/ml and adherent cells were approximately 85% confluent at the time of transfection.



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TransIT-VirusGEN® SELECT Transfection Reagent

The *Trans*IT-VirusGEN[®] SELECT Transfection Reagent is designed for large-scale virus production in preclinical and early-phase clinical trials and is identical in formulation to the fully synthetic and animal origin free, research-grade *Trans*IT-VirusGEN[®] Transfection Reagent. *Trans*IT-VirusGEN[®] SELECT is tested for performance, identity, sterility, endotoxin and mycoplasma to streamline ancillary material qualification in viral vector manufacturing.

- Performance Efficient DNA delivery for large-scale production of high-titer viral vectors
- Quality Tested for performance, identity, sterility, endotoxin and mycoplasma
- Reliability Exceptional lot-to-lot consistency
- Flexibility Compatible with different virus production platforms and repeat filtration

Compare <i>Trans</i> IT-VirusGEN® Solutions	Research & Development	Preclinical & Early-phase Clinical Trial TransIT-VirusGEN® SELECT	Late-pha Clinical Tr & Commerc Manufactu TransIT-VirusGEN	rial :ial ring
Composition	IDENTICAL <i>Trans</i> IT-VirusGEN® REAGENT FORMULATION Ready-to-use, chemically defined, animal origin free			
Quality	R&D	Preclinical	GMP	
Quality Control	Standard Functional QC	Sterility: per USP <71> Bacterial Endotoxin: per USP <85> Mycoplasma: per USP <63> Formulation Identity	Manufactured as a assured product ac relevant guidelines Manufacturing Prac	cording to for Good
Configuration	0.3 ml; 0.75 ml; 1.5 ml; 5x and 10x1.5 ml	30 ml 150 ml	150 ml	
Packaging	Vial	Bottle	Bottle	
Availability	Purchase or Sample: www.mirusbio.com/sample	Purchase or Sample: www.mirusbio.com/sample	2021	
Product Description			PRODUCT NO.	QUANTITY
TransIT-VirusGEN® SELECT Designed to enhance delivery of viral vector DNA to suspension and adherent HEK 293 cells for high-titer production of recombinant lentivirus and adeno-associated virus (AAV). Includes quality documentation. Ideal for large- scale virus production and early-phase clinical trials. MIR 6730 30				30 ml
		NEW!	MIR 6735	150 ml

Request a FREE sample of TransIT-VirusGEN® Transfection Reagent: www.mirusbio.com/SELECT

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