

HTRANSFECTION | Virus Production

NOW Available! 150 ml size

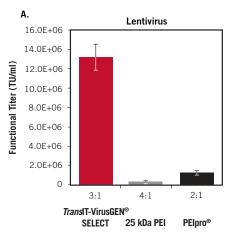


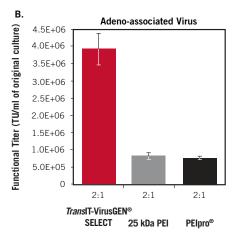




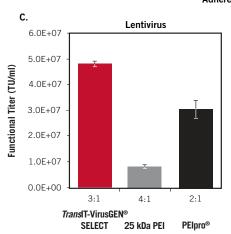
TransIT-VirusGEN® SELECT Outperforms PEI and PEIpro®

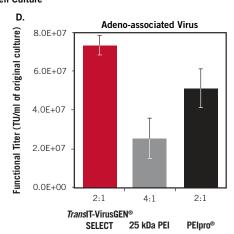






Adherent Cell Culture





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 PElpro* (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.*

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

Product Name: TransIT-VirusGEN® SELECT Transfection Reagent

Product Number: MIR 6730
Lot Number:

Animal Derived Components: None

Type of Manufacture: Chemical Synthesis
Country of Manufacture: USA

This information is to be used for the purpose of determining animal origin only and not to be confused with "country of origin" for import/export purposes.

BSE TSE Statement



Product Name: TransIT-VirusGEN® SELECT Transfection Reagent

Product Number: MIR 6730

Lot Number: Issue Date:

This product is manufactured in Madison, WI (USA) entirely from material of non-animal origin. The manufacture, packaging, storage and transportation of these materials do not involve the use of material of animal origin. Therefore, these products have minimal risk of contamination with Bovine Spongiform Encephalopathy (BSE) or Transmissible Bovine Encephalopathy (TSE).

Mirus Bio LLC does not have plans to change the production of this material in any way that would increase the risk of BSE or TSE contamination.

Certified By: Juan

Susan Elder, Quality Cont This product is sold to the Buyer wi

Rev. Example

Mirus Bio LLC | 560

Signed on behalf of Mirus Bio LLC By:

Susan Elde

Susan Elder, Quality Superviso

 $\textbf{Mirus Bio LLC} \ | \ 5602 \ Research \ Park \ Blvd. \ Ste \ 210 \ | \ Madison, \ Wisconsin \ 53719 \ USA \ | \ 608.441.2852 \ | \ FAX \ 608.441.2849 \ | \ www.mirusbio.com \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ | \ 100 \ |$

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

Mirus.

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 Assay1	> 500,000 TU/mL	Pass
Sterility Testing ²	No growth observed	Pass
Endotoxin Testing ³	≤1 EU/mL	Pass
Mycoplasma Testing4	None detected	Pass
Identity	All components detected	Pass
Appearance	Clear, colorless solution	Pass

References

¹TransIT-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 Eldn

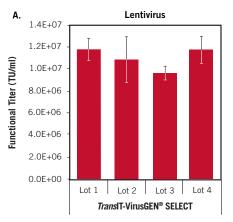
Susan Elder, Quality Supervisor

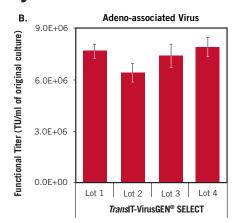
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Reliable Lot-to-Lot Consistency

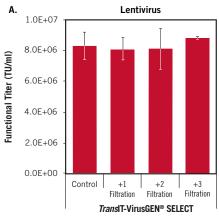


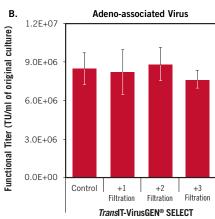


(A) Lentivirus was produced using suspension FreeStyle™ 293-F cells grown in FreeStyle™ F17 Medium and transfected with 3rd generation vectors pLKO.1-puro-CMV-TurboGFP™ transfer vector (Sigma) and ViraSafe™ Pantropic Packaging mix (pRSV-Rev, pCMV-VSV-G, pCgpV, Cell Bio Labs) at a 3:0.5:0.5:2 DNA ratio, 1 ug/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 ug/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.*



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>Tran</i> sIT-VirusGEN®	Research &	Preclinical &	Late-ph Clinical &		
Solutions	Development	Early-phase Clinical Trial	Comme Manufact		
	TransIT-VirusGEN®	TransIT-VirusGEN® SELECT	<i>Trans</i> IT-VirusG	EN® GMP	
Composition	IDENTICAL TransIT-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 quality- assured product according to relevant guidelines for Good Manufacturing Practice (GMP)		
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 Transfection Reagent	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.				
		NEW!	MIR6735	150 ml	

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