VirusGEN® AAV Transfection Kit with *Rev*IT™ AAV Enhancer

Quick Reference Protocol

Instructions for MIR 8007, 8008

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/8007

SPECIFICATIONS

Storage	Store *Trans*IT-VirusGEN® Transfection Reagent and *Rev*IT™ AAV Enhancer at -10 to -30°C, tightly capped. ***Before each use***, warm to room temperature and vortex gently. ***Do not freeze/thaw Rev*IT™ AAV Enhancer > *5 times***.
Product Guarantee	When properly stored and handled, *Trans*IT-VirusGEN® Transfection Reagent is guaranteed for 1 year from the date of purchase, and *Rev*IT™ AAV Enhancer is guaranteed for 6 months from the date of purchase.

PROTOCOL FOR ADENO-ASSOCIATED VIRUS (AAV) GENERATION IN SUSPENSION HEK 293 CULTURES

Full protocol and additional documentation available at ***mirusbio.com/8007***

VirusGEN® AAV Transfection Kit with *Rev*IT™ AAV Enhancer Workflow

A. Maintain Cells

Passage cells regularly and ensure they are >95% viable before transfection.

B. Day 0

Dilute cells to **3 × 10^6** cells/ml.

Form transfection complexes in PBS ***or*** basal cell culture medium; use a volume that is 5-10% of cell culture volume.

Per ml of culture, add
1. **DNA:** 1 - 2 µg
2. ***Rev*IT™:** 0.5 - 1.5 µl
3. ***Trans*IT-VirusGEN®:** 1.5 - 3 µl.

Incubate stationary for **15 - 45 min**.

C. Add transfection complexes to cells.

D. Day 2 - 3

Harvest AAV **48 - 72 hr** post-transfection.

Total Plasmid DNA refers to the combined mass of packaging plasmids and the transfer plasmid containing the gene-of-interest. Premix the plasmids together prior to adding to the complex formation medium.

Fill in volumes below based on total culture volume (Table 1).

A. Maintain cells

1. Passage suspension HEK 293 cells 18-24 hours prior to transfection to obtain a density of 3 - 4 × 10^6 cells/ml the next day. Do NOT proceed with transfection if cells are not doubling every 24 hours or are < 95% viable.
2. Incubate cells overnight at appropriate temperature and CO_2 levels.

B. Prepare *Trans*IT-VirusGEN® Reagent:*Rev*IT™ AAV Enhancer:DNA complexes

1. At time of transfection, seed cells to a density of 3 × 10^6 cells/ml.
2. Warm *Trans*IT-VirusGEN® Reagent to room temperature and vortex.
3. Place ___ml of PBS or basal serum-free cell culture media in a sterile tube.
4. Add ___µl of the total plasmid DNA to the tube. Mix gently by pipetting.
5. Add ___µl of *Rev*IT™ AAV Enhancer. Mix completely.
6. Add ___µl of *Trans*IT-VirusGEN® Reagent. Vortex gently to mix.
7. Incubate at room temperature for 15-45 minutes to allow transfection complexes to form. Do not vigorously agitate complexes again once formed.

C. Distribute complexes to cells in complete growth medium

1. Add *Trans*IT-VirusGEN® Reagent:*Rev*IT™ AAV Enhancer:DNA complexes (from Step B) to cells.
2. Incubate cultures in appropriate conditions (i.e. 37°C, 5 - 8% CO_2, shaking) for 48-72 hours prior to AAV harvest.

D. Harvest virus

1. Following the 48-72 hour incubation, prepare 10X Cell Lysis Buffer (500 mM Tris pH 8, 10% Tween® 20, 20 mM $MgCl_2$).
2. Transfer the total volume of cell suspension (___ml) to a sterile conical tube or appropriate vessel.
3. Add 0.1X volume (___ml) of 10X Cell Lysis Buffer and 100 U/ml (___µl) of Benzonase®. Mix completely and incubate at 37°C for 1.5 hours with shaking.
4. Add 0.1X volume (___ml) of 5 M NaCl. Mix completely and incubate at 37°C for 30 minutes with shaking.
5. Centrifuge the mixture at 4,100 × g for 10 minutes to remove cell debris.
6. Transfer the AAV-containing supernatant to a new tube. Store at -80°C.

Table 1. Volume scaling worksheet for VirusGEN® AAV Transfection Kit with *Rev*IT™

Starting conditions per milliliter of complete growth medium					
	Per 1 ml		Total culture volume		Reagent quantities
PBS or Serum-free Basal Medium	0.1 ml	×	_____ml	=	_____ml
Total Plasmid DNA (1 µg/µl)	2 µl	×	_____ml	=	_____µl
*Trans*IT-VirusGEN® Reagent	3 µl	×	_____ml	=	_____µl
*Rev*IT™ AAV Enhancer	1 µl	×	_____ml	=	_____µl

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NOTES

Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at: mirusbio.com/ra

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