

TransIT®-AAViator Transfection System

Quick Reference Protocol

Instructions for MIR 73750 and MIR 73745

SDS and Certificate of Analysis available at mirusbio.com/literature



SPECIFICATIONS

Storage	Store TransIT®-AAViator Transfection Reagent and RevIT™ AAV Enhancer at -10 to -30°C, tightly capped. Before each use , warm to ambient temperature greater than 19°C and vortex gently. RevIT™ AAV Enhancer is known to maintain function through at least five freeze-thaw cycles (thawed in a 37°C incubator). Return to proper storage conditions after each use.
Product Guarantee	When properly stored and handled, TransIT®-AAViator Transfection Reagent and RevIT™ AAV Enhancer are guaranteed for 6 months from the date of purchase.

TransIT®-AAViator Transfection System Workflow

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

Thaw RevIT™
For best results, RevIT™ AAV Enhancer is intended to be used with TransIT®-AAViator Transfection Reagent.

Day 0
Seed cells.

Form transfection complexes in basal cell culture medium; use a volume that is 5% of cell culture volume.

Per ml of culture, add
1. **DNA:** 1.0 - 1.5 µg
2. **RevIT™:** 0.5 - 1.5 µl
3. **TransIT®-AAViator:** 1.0 - 2.3 µl

Incubate stationary for 15 - 45 min.

Add transfection complexes to cells.

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 $3 - 4 \times 10^6$ 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 CO₂ levels.
- B. Prepare TransIT®-AAViator Reagent:RevIT™ AAV Enhancer:DNA complexes**
1. At time of transfection, seed cells to a density of 3×10^6 cells/ml.
 2. Warm TransIT®-AAViator Transfection Reagent and RevIT™ AAV Enhancer to room temperature and vortex. If thawing at room temperature, allow ~4 hours and ensure ambient air temperature is > 19°C.
 3. Place ____ ml of 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 RevIT™ AAV Enhancer. Mix completely.
 6. Add ____ µl of TransIT®-AAViator Transfection Reagent. Vortex gently to mix.
 7. Incubate at room temperature for 15-45 minutes to allow transfection complexes to form. Do not vigorously agitate or vortex complexes again after incubation.
- C. Distribute complexes to cells in complete growth medium**
1. Add TransIT®-AAViator:RevIT™ AAV Enhancer:DNA complexes (from Step B) to cells in a uniform manner.
 2. Incubate cultures in appropriate conditions (i.e. 37°C, 5-8% CO₂, 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 MgCl₂).
 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 $4,100 \times g$ 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 TransIT®-AAViator Transfection System

Starting conditions per milliliter of complete growth medium			
	Per 1 ml	Total culture volume	Reagent quantities
Serum-free Basal Medium	0.05 ml	× _____ ml	= _____ ml
Total Plasmid DNA (1 µg/µl)	1.5 µl	× _____ ml	= _____ µl
RevIT™ AAV Enhancer	1.0 µl	× _____ ml	= _____ µl
TransIT®-AAViator Reagent	1.9 µl	× _____ ml	= _____ µl

For Research Use Only

Mirus Bio LLC

www.mirusbio.com | techsupport@mirusbio.com | U.S. Toll Free: 844.647.8724 | Direct: +1.608.441.2852



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

©1996-2025 All rights reserved. Mirus Bio LLC. All trademarks are the property of their respective owners.
For terms and conditions, visit www.mirusbio.com

Rev1 02OCT2024

Mirus Bio LLC

www.mirusbio.com | techsupport@mirusbio.com | U.S. Toll Free: 844.647.8724 | Direct: +1.608.441.2852