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

Now With VirusGEN® Transfection Complex Stabilizer

Protocol for MIR 8007, MIR 8008, MIR 6770, MIR 6773 and MIR 6775.

Quick Reference Protocol, SDS and CoA available at mirusbio.com/literature

INTRODUCTION

Adeno-associated virus (AAV) is a nonenveloped, single-stranded DNA virus from the *Parvoviridae* family notable for its lack of pathogenicity, low immunogenicity and ability to infect both dividing and quiescent cells. Because AAV is replication-defective in the absence of adenovirus or helper proteins and is not implicated in any known human diseases, it is widely considered a safe gene delivery vehicle for *in vivo* and *in vitro* applications. Accordingly, recombinant AAV has become an invaluable tool for gene therapy and the creation of isogenic human disease models.

The VirusGEN® AAV Transfection Kit with *Rev*IT™ AAV Enhancer part numbers MIR 8007 and MIR 8008 include:

- *Trans*IT-VirusGEN® Transfection Reagent proven for AAV production in suspension cells.
- *Rev*IT™ AAV Enhancer designed to increase AAV titer 2-4X for any serotype, reagent, HEK293 cell line or media.
- VirusGEN® Transfection Complex Stabilizer, which extends transfection complex formation time by up to 3 hours and reduces transfection complex volume down to 2% of total culture volume, while maintaining high titers and percent full AAV capsids.

Please refer to the workflow diagram on page 3 and the 'Using VirusGEN® Transfection Complex Stabilizer' section on page 4 for how to use it in your workflow.

SPECIFICATIONS

Storage	Store *Trans*IT-VirusGEN® Transfection Reagent, *Rev*IT™ AAV Enhancer and VirusGEN® Transfection Complex Stabilizer at -10 to -30°C, tightly capped. **Before each use**, warm to room temperature and vortex gently. *Rev*IT™ AAV Enhancer 1.5 ml takes approximately 4 hours to thaw at room temperature, and 30 minutes at 37°C. *Rev*IT™ 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.
Stability / Guarantee	When properly stored and handled, *Trans*IT-VirusGEN® Transfection Reagent is guaranteed for 1 year from date of purchase. *Rev*IT™ AAV Enhancer and VirusGEN® Transfection Complex Stabilizer are both guaranteed for 6 months from date of purchase.

Warm all reagents to room temperature and mix gently before each use.

NOTE: *Rev*IT™ AAV Enhancer remains frozen at temperatures < 19°C.

MATERIALS

The VirusGEN® AAV Transfection Kit with *Rev*IT™ AAV Enhancer is supplied in ***one*** of the following formats:

Product No.	Volume of *Trans*IT-VirusGEN® Transfection Reagent	Volume of VirusGEN® Transfection Complex Stabilizer	Volume of *Rev*IT™ AAV Enhancer
MIR 8007	2 × 1.5 ml	2 × 150 µl	1 × 1.5 ml
MIR 8008	1 × 30 ml	2 × 1.5 ml	10 × 1.5 ml

BEFORE YOU START:

Important Tips for Optimal AAV Production

The suggestions below yield high-efficiency plasmid DNA transfection using the VirusGEN® AAV Transfection Kit with *Rev*IT™ AAV Enhancer and the VirusGEN® Transfection Complex Stabilizer.

- **Cell culture conditions.** Use suspension HEK 293 cells with the VirusGEN® AAV Transfection Kit with *Rev*IT™ AAV Enhancer. Before transfection, ensure cells are ≥ 95% viable by trypan blue exclusion and doubling every 24 hours. After transfection, there is no need to perform a medium change to remove the transfection complexes.
- **Cell density at transfection.** The recommended cell density is 3 × 10^6 cells/ml. Passage cells 18-24 hours before transfection to ensure that cells are actively dividing and reach the appropriate density at time of transfection.
- **AAV packaging and transfer plasmids.** The optimal ratio between plasmids will depend on the vector backbone and gene-of-interest. For each unique construct, empirically determine and use the optimal ratio for best results. Use plasmid manufacturer recommendations or previously established ratios as a starting point.
- ***Rev*IT™ AAV Enhancer.** Titrate *Rev*IT™ AAV Enhancer from 0.5 to 1.5 µl per 1 ml of culture to determine the optimal amount for production of your specific viral vector.
- **Ratio of *Trans*IT-VirusGEN® to DNA.** Determine the optimal *Trans*IT-VirusGEN® Reagent:DNA ratio for each cell type by varying the amount of reagent. Refer to **Table 1 or 2** for recommended starting conditions based on culture size.
- **Complex formation conditions.** Prepare *Trans*IT-VirusGEN® Reagent (supplemented with VirusGEN® Stabilizer):*Rev*IT™ AAV Enhancer:DNA complexes in PBS in a volume that is 2-5% of the total culture volume.
- **VirusGEN® Transfection Complex Stabilizer.** Premix the VirusGEN® Transfection Complex Stabilizer with the *TransIT*-VirusGEN® Transfection Reagent prior to adding to the complex formation medium. Due to differences in AAV processes, it is recommended to titrate the amount of VirusGEN® Transfection Complex Stabilizer; see an example optimization experiment detailed in Table 3 and 4 on page 8.

Premix VirusGEN® Transfection Complex Stabilizer and *Trans*IT-VirusGEN® Transfection Reagent together prior to adding to the complex formation medium.

Premix packaging and transfer plasmids together prior to adding to the complex formation medium.

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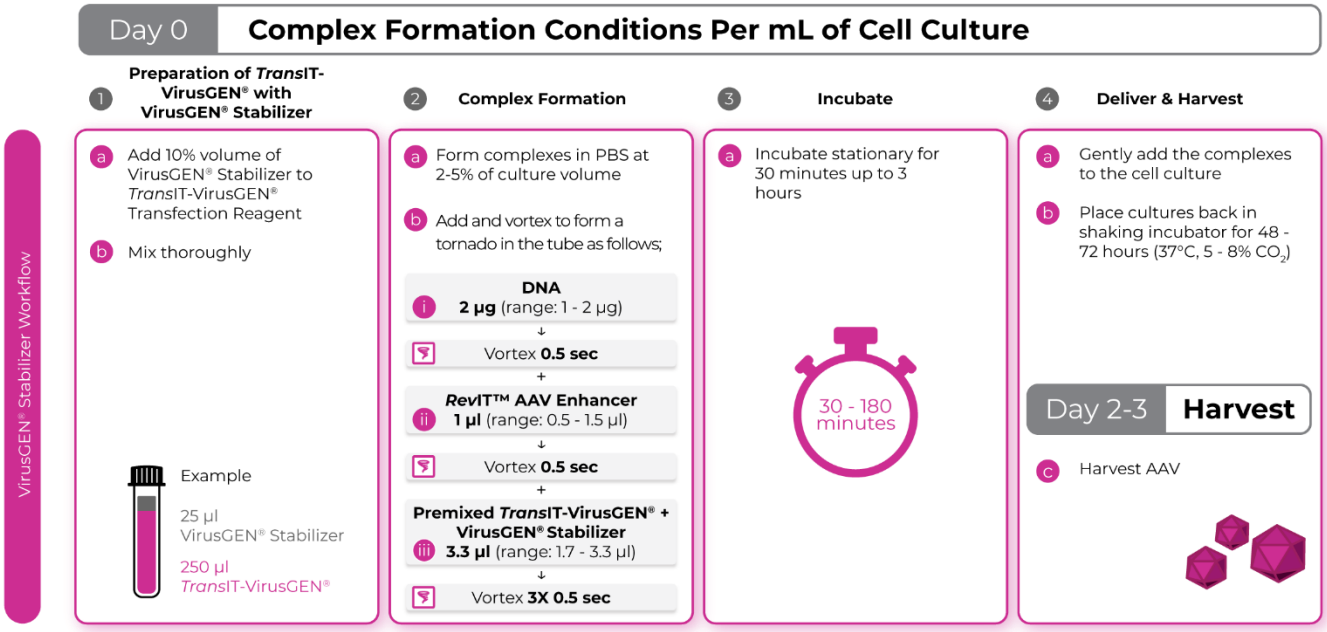
Materials Required, But Not Supplied

- Suspension HEK 293 Cells (e.g. Viral Production Cells 2.0, Gibco Cat. No. A49784)
- Complete Culture Medium (e.g. Viral Production Medium, (Gibco Cat. No. A4817901) or CellVento 4HEK (Millipore Sigma Cat. No. 125193)). CellVento 4HEK requires supplementation with 6mM L-glutamine (MIR 6240).
- Plasmid DNA (e.g. pAAV-hrGFP (Agilent Cat. No. 240074-51), pHelper (Agilent Cat. No. 240071-54), AAV8 Rep-Cap Plasmid (GeneMedi Cat. No. P-RC09))
- Phosphate Buffered Saline (PBS) (e.g. Millipore Sigma Cat. No. D8537)
- Erlenmeyer shake flasks (e.g. Corning® Cat. No. 431143 or Thomson Cat. No. 931110)
- 10X Cell Lysis Buffer (500 mM Tris pH 8, 10% Tween® 20, 20 mM $MgCl_2$)
- 5 M Sodium Chloride (5 M NaCl)
- Benzonase® or equivalent (e.g. Millipore Sigma Cat. No. E1014 or Syd Labs Cat. No. BP4200)

Workflow: VirusGEN® AAV Transfection Kit with *Rev*IT™ AAV Enhancer & VirusGEN® Transfection Complex Stabilizer



Using VirusGEN® Transfection Complex Stabilizer

Using the VirusGEN® Transfection Complex Stabilizer increases the complex formation time from 30 minutes to up to three hours, allowing for increased time to complete transfection successfully. It also allows for smaller complex formation volumes, down to 2-5% of total culture volume.

To use the VirusGEN® Transfection Complex Stabilizer, add 10% volume to *Trans*IT-VirusGEN® prior to forming transfection complexes i.e. add 25 µl VirusGEN® Stabilizer to 250 µl *Trans*IT-VirusGEN® and mix well. When using *Trans*IT-VirusGEN® supplemented with VirusGEN® stabilizer, extra volume of reagent should be added to the transfection complex to compensate, see Table 1 or 2 on Page 5. The extra amount needed to compensate corresponds to the percent volume of Stabilizer added i.e. if 10% volume of VirusGEN® stabilizer is added, 10% extra supplemented *Trans*IT-VirusGEN® should be added when forming the transfection complex.

Mirus recommends first testing a range of concentrations of VirusGEN® Transfection Complex Stabilizer for your workflow, see the optimization steps detailed in Table 3 and 4 on page 8.

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AAV GENERATION IN SUSPENSION HEK 293 CELL CULTURES

NOTE: Use of the VirusGEN® AAV Transfection Kit with *Rev*IT™ AAV Enhancer is only recommended for AAV production in suspension HEK 293 cell lines. Contact Mirus Bio Technical Support (techsupport@mirusbio.com) for optimization in adherent cell culture platforms.

Scale the amounts of serum-free complex formation medium, *Trans*IT-VirusGEN® Reagent, *Rev*IT™ AAV Enhancer and total DNA based on the **total culture volume** to be used. To calculate the required reagent quantities based on the recommended starting conditions and total culture volume, refer to **Table 1** or **Table 2.**

NOTE: VirusGEN® Transfection Complex Stabilizer enables the use of low complex formation volumes, at either 5% or 2% of total culture volume. Use Table 1 or 2 depending on your workflow. An optimization experiment for determining the best concentration of VirusGEN® Transfection Complex Stabilizer is detailed in Table 3 and 4 on page 8.

Total plasmid DNA refers to the combined weight of AAV plasmids (in µg) per transfection.

Table 1. Calculation worksheet for scaling *Trans*IT-VirusGEN® + VirusGEN® Stabilizer with *Rev*IT™ AAV Enhancer

Workflow for Forming Complexes at 2% of Total Culture Volume					
Starting conditions per milliliter of complete growth medium					
	Per 1 ml		**Total Culture Volume**		**Reagent Quantities**
PBS	13.7 µl	×	ml	=	ml
Total Plasmid DNA (1 µg/µl stock)	2 µl	×	ml	=	µl
*Rev*IT™ AAV Enhancer	1 µl	×	ml	=	µl
*Trans*IT-VirusGEN® (with VirusGEN® Stabilizer)	3.3 µl	×	ml	=	µl

Vortex 0.5 sec after addition of DNA and *Rev*IT™ AAV Enhancer, and **3x 0.5 sec** after all reagents are combined, forming a tornado in the tube. For larger scale, see note on page 7 about using 2D bioprocessing bags.

Table 2. Calculation worksheet for scaling *Trans*IT-VirusGEN® + VirusGEN® Stabilizer with *Rev*IT™ AAV Enhancer

Workflow for Forming Complexes at 5% of Total Culture Volume					
Starting conditions per milliliter of complete growth medium					
	Per 1 ml		**Total Culture Volume**		**Reagent Quantities**
PBS	43.7 µl	×	ml	=	ml
Total Plasmid DNA (1 µg/µl stock)	2 µl	×	ml	=	µl
*Rev*IT™ AAV Enhancer	1 µl	×	ml	=	µl
*Trans*IT-VirusGEN® (with VirusGEN® Stabilizer)	3.3 µl	×	ml	=	µl

Transient Transfection Protocol

Use Table 1 or Table 2 on Page 5 to calculate the required volumes for your workflow.

A. Maintenance of cells

1. Passage suspension HEK 293 cells 18-24 hours prior to transfection to ensure that cells are actively dividing at the time of transfection and to obtain a density of 3 - 4 ×10^6 cells/ml the next day.
   NOTE: Perform cell counts and evaluate viability daily to ensure that cells are doubling every 24 hours and ≥ 95% viable by trypan blue exclusion. Do NOT proceed with transfection if cells are not doubling normally or are < 95% viable.
2. Incubate cells overnight under appropriate conditions (e.g. 37°C, 5-8% CO_2, shaking).

Divide cultured cells 18-24 hours before transfection to ensure that the cells are actively dividing at the time of transfection.

B. Prepare *Trans*IT-VirusGEN®:*Rev*IT™ AAV Enhancer:DNA complexes (immediately before transfection)

1. Warm *Trans*IT-VirusGEN® Reagent, VirusGEN® Transfection Complex Stabilizer and *Rev*IT™ AAV Enhancer to room temperature and vortex gently before using. *Rev*IT™ AAV Enhancer can be incubated in an incubator that is set to 37°C to accelerate thawing. Allow 30 minutes at 37°C, or 4 hours at room temperature.
2. Add the VirusGEN® Transfection Complex Stabilizer to *Trans*IT-VirusGEN® Reagent at 10% volume i.e. add 25 µl Stabilizer to 250 µl of Reagent and mix thoroughly.
3. Immediately prior to transfection, seed cells at a density of 3 × 10^6 cells/ml into a transfection culture vessel (e.g. 30 ml into a 125 ml Erlenmeyer shake flask).
4. Place ___ ml of PBS in a sterile tube.
5. In a separate sterile tube, combine AAV plasmids per manufacturer recommendations to a final concentration of 1 µg/µl. Mix thoroughly.
6. Transfer ___ µl of the DNA mixture prepared in Step B.5 to the tube containing PBS. Vortex with a 0.5 second pulse.
7. Add ___ µl of *Rev*IT™ AAV Enhancer to the diluted DNA and PBS. Vortex with a 0.5 second pulse.
8. Add ___ µl of *Trans*IT-VirusGEN® Reagent (+ VirusGEN® Stabilizer) to the diluted DNA:*Rev*IT™ mixture. Mix completely by vortexing for three 0.5 second pulses to form a tornado in the tube. Do NOT agitate *Trans*IT-VirusGEN®:*Rev*IT™:DNA complexes again after this initial mixing.
   **For customers working at large scale, see note about mixing on page 7.*
9. Incubate at room temperature for 30 minutes or up to 180 minutes without additional agitation to allow transfection complexes to form.

Do NOT allow the *Trans*IT-VirusGEN® Reagent to incubate alone in complex formation solution > 5 minutes, i.e. if the reagent is pre-diluted, add DNA within 5 minutes for optimal complex formation.

Do NOT agitate *Trans*IT-VirusGEN®:*Rev*IT™:DNA complexes after the initial mixing. This will result in decreased titer.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-VirusGEN®:*Rev*IT™:DNA complexes (prepared in Step B) to culture vessel, swirling gently to distribute.
2. Shake flasks on an orbital shaker (125 rpm when using a shaker with a 1.9 cm orbital throw) at appropriate temperature and CO_2 levels (e.g. 37°C, 5-8% CO_2).
3. Incubate cultures for 48-72 hours prior to AAV harvest.

It is recommended to test with and without the VirusGEN® Stabilizer, and to optimize the concentration. See Table 3 & 4 on page 8 for an optimization experiment.

D. Harvest and storage of AAV

1. Following the 48-72 hour incubation, transfer the total volume of cell suspension (i.e. 33 ml) to a sterile conical tube or appropriate vessel.
2. Add 0.1X volume of 10X Cell Lysis Buffer (i.e. 3.3 ml for 30 ml culture) and 100 U/ml Benzonase® (i.e. 3,300 U). Mix completely and incubate at 37°C for 1.5 hours with shaking.
3. Add 0.1X volume of 5 M NaCl (i.e. 3.3 ml for 30 ml culture) and mix completely. Incubate at 37°C for 30 minutes with shaking.
4. Centrifuge the mixture at 4,100 × g for 10 minutes to remove cell debris. Carefully transfer the AAV containing supernatant to a new sterile tube.
5. Store AAV stocks at -80°C.

Benzonase® is a non-specific endonuclease used to liberate virus particles from residual nucleic acids in the cell lysates and increase AAV titers.

*__For customers working at large scale,__ where vortexing of transfection components is unfeasible, Mirus recommends either mixing by inversion or using a rocking platform. Fill a vessel, e.g. 2D bioprocessing bag, with PBS. Ensure the size of the vessel has enough headspace to allow for effective mixing. Add the DNA and RevIT™ AAV Enhancer, mixing well after each addition. Then, add the *Trans*IT-VirusGEN® Transfection Reagent (+ VirusGEN® Transfection Complex Stabilizer) directly to the mixture. Mixing can be performed by inversion (10-20 times) or rocking gently on a rocking platform for 5-7 seconds (e.g. 25 rpm at a 12° angle). After mixing, allow the transfection complexes to form by incubating stationary. Near the end of the desired incubation time, begin gravity draining or pumping the transfection complexes into the bioreactor. Complexes can be effectively pumped at speeds of 1-2 L/min through #73 tubing (3/8" inner diameter); if using a different size of tubing, adjust the flow rate accordingly. Please contact techsupport@mirusbio.com for additional guidance.

Optimizing VirusGEN® Transfection Complex Stabilizer

Mirus recommends optimization of the concentration of VirusGEN® Transfection Complex Stabilizer to achieve the highest yields in your system, whilst achieving the desired incubation time point. Table 3 and 4 detail an example evaluation grid to determine which is the best concentration to use for 30 ml culture in a 125 ml Erlenmeyer shake flask. If using an alternative culture volume, adjust the components using Table 1 and 2 on Page 5. A 90-minute incubation time is used in this example, but the VirusGEN® Transfection Complex Stabilizer shows effective performance when transfection complexes are incubated for a minimum of 30 minutes, up to a maximum of 180 minutes.

To adjust the concentration of VirusGEN® Stabilizer, reduce or increase the amount added to *Trans*IT-VirusGEN® prior to complex formation. i.e. for 0.8X VirusGEN® Stabilizer, add 8% volume (20 µL in 250 µL).

Table 3. Evaluation Grid for VirusGEN® Transfection Complex Stabilizer for 30 ml culture, forming complexes at 2% of total culture volume.

Condition	Stabilizer Concentration	% Complex Formation Volume	PBS	pDNA (1mg/ml)	*Rev*IT™ AAV Enhancer	Transfection Reagent Volume	Volume added to culture	Incubation Time
Control 30 min	-	5%	1.31 ml	60 µl	30 µl	90 µl	1.5 ml	30 min
Control 90 min	-	5%	1.31 ml	60 µl	30 µl	90 µl	1.5 ml	180 min
0.8X Stabilizer 30 min	0.8X	2%	413 µl	60 µl	30 µl	97 µl	600 µl	30 min
0.8X Stabilizer 180 min	0.8X		413 µl	60 µl	30 µl	97 µl	600 µl	180 min
1x Stabilizer 30 min	1X		411 µl	60 µl	30 µl	99 µl	600 µl	30 min
1x Stabilizer 180 min	1X		411 µl	60 µl	30 µl	99 µl	600 µl	180 min
1.2X Stabilizer 30 min	1.2X		409 µl	60 µl	30 µl	101 µl	600 µl	30 min
1.2X Stabilizer 180 min	1.2X		409 µl	60 µl	30 µl	101 µl	600 µl	180 min

Table 4. Evaluation Grid for VirusGEN® Transfection Complex Stabilizer for 30 ml culture, forming complexes at 5% of total culture volume.

Condition	Stabilizer Concentration	% Complex Formation Volume	PBS	pDNA (1mg/ml)	*Rev*IT™ AAV Enhancer	Transfection Reagent Volume	Volume added to culture	Incubation Time
Control 30 min	-	5%	1.31 ml	60 µl	30 µl	90 µl	1.5 ml	30 min
Control 180 min	-	5%	1.31 ml	60 µl	30 µl	90 µl	1.5 ml	180 min
0.8X Stabilizer 30 min	0.8X	5%	1.31 ml	60 µl	30 µl	97 µl	1.5 ml	30 min
0.8X Stabilizer 180 min	0.8X		1.31 ml	60 µl	30 µl	97 µl	1.5 ml	180 min
1x Stabilizer 30 min	1X		1.31 ml	60 µl	30 µl	99 µl	1.5 ml	30 min
1x Stabilizer 180 min	1X		1.31 ml	60 µl	30 µl	99 µl	1.5 ml	180 min
1.2X Stabilizer 30 min	1.2X		1.31 ml	60 µl	30 µl	101 µl	1.5 ml	30 min
1.2X Stabilizer 180 min	1.2X		1.31 ml	60 µl	30 µl	101 µl	1.5 ml	180 min

Reach out to techsupport@mirusbio.com for help with optimizing the VirusGEN® Transfection Complex Stabilizer.

TROUBLESHOOTING GUIDE

POOR DNA TRANSFECTION EFFICIENCY	
Problem	**Solution**
Incorrect vector sequence	If you do not observe expression of your target insert, verify the sequence of your plasmid DNA.
Suboptimal *Trans*IT® Reagent:*Rev*IT™:DNA ratio	Determine the best *Trans*IT-VirusGEN® Reagent:*Rev*IT™ AAV Enhancer:DNA ratio for each cell type. Titrate the *Trans*IT-VirusGEN® Reagent volume from 1-3 µl per 1 µg of DNA. Titrate the *Rev*IT™ AAV Enhancer volume from 0.5-1.5 µl per 1 ml of culture. Refer to "Before You Start" on Page 2 for recommended starting conditions.
Suboptimal VirusGEN® Stabilizer Concentration	Determine the best concentration of VirusGEN® Transfection Complex Stabilizer for your process. Refer to Table 3 & 4 on page 8 for an example optimization experiment, whereby different concentrations of VirusGEN® Transfection Complex Stabilizer, such as 1.2X, 1X or 0.8X, should be tested. If too much VirusGEN® Transfection Complex Stabilizer is used, titers at the short complex formation time point may be lower than maximum; if too little VirusGEN® Transfection Complex Stabilizer is used, titers at the long complex formation time may be lower than maximum. It is also possible with the addition of excess VirusGEN® Transfection Complex Stabilizer that both the short and long timepoints yield lower than maximum titers.
Incorrect addition of VirusGEN® Stabilizer	Add VirusGEN® Stabilizer to *Trans*IT-VirusGEN® at 10% volume (i.e. 25 µl to 250 µl) and mix well by vortexing prior to complex formation.
Suboptimal DNA concentration	Determine the DNA concentration accurately. Use plasmid DNA preps with an $A_{260/280}$ of 1.8-2.0.
	The optimal DNA concentration generally ranges between 0.5-2 µg per 1 ml of culture. Start with 2 µg DNA per 1 ml of culture. Consider testing different amounts of DNA while scaling the amount of *Trans*IT-VirusGEN® accordingly.
Low-quality plasmid DNA	Use highly purified, sterile, endotoxin- and contaminant-free DNA for transfection.
	We recommend using Mirus MiraCLEAN® Endotoxin Removal Kit (MIR 5900) for removal of endotoxin from your DNA preparation. Alternatively, use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells.
Cells not actively dividing at the time of transfection	Divide the culture at least 18-24 hours before transfection to ensure that the cells are actively dividing and reach optimal cell density at time of transfection. DO NOT proceed with transfection if cells are not doubling normally or are < 95% viable by trypan blue exclusion.
Time of AAV harvest not optimal	Determine the optimal time to harvest AAV post-transfection. Though typically 48-72 hours post-transfection, the best time to harvest will depend on the vector construct and production platform.
*Trans*IT-VirusGEN® was not mixed properly	Warm *Trans*IT-VirusGEN® Reagent to room temperature and vortex gently before each use.
	If *Trans*IT-VirusGEN® Reagent is pre-diluted in complex formation solution, DNA should be added within 5 minutes. Incubating the *Trans*IT-VirusGEN® Reagent in complex formation solution alone for an extended time results in reduced production of functional virus.
Disruption of transfection complex formation	After initial mixing of DNA, *Rev*IT™ AAV Enhancer and *Trans*IT-VirusGEN® Reagent (+ VirusGEN® Stabilizer), do not agitate the Reagent:Enhancer:DNA complexes again during or after the incubation period, e.g. do not vortex or invert before adding to cultures.
Precipitate formation during transfection complex formation	During complex formation, scale all reagents according to the table in the protocol, including serum-free media, *Trans*IT-VirusGEN® Reagent (+/- VirusGEN® Stabilizer), *Rev*IT™ AAV Enhancer, and/or plasmid DNA.
	Precipitation may be observed when excess DNA is used during complex formation. This may negatively impact transfection efficiency. To avoid precipitation when using high concentrations of DNA, increase the volume of serum-free medium during complex formation by two-fold.
	Large-volume transfection complexes may appear turbid – typically, this phenomenon does *not* negatively impact transfection as long as complexes are well mixed.
Verify Transfection Efficiency	To assess delivery efficiency of plasmid DNA, use Mirus *Label* IT® Nucleic Acid Labeling Kits to label the target plasmid or use Mirus prelabeled *Label* IT® Plasmid Delivery Controls (please refer to "Related Products" on Page 10).
	To verify efficient transfection, use *Trans*IT-VirusGEN® Reagent to deliver a positive control such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid.

TROUBLESHOOTING GUIDE CONTINUED

HIGH CELLULAR TOXICITY	
Problem	**Solution**
Cell density not optimal at time of transfection	High toxicity and cell death may be observed if cells are not dense at the time of transfection. For high virus titers using *Trans*IT-VirusGEN® Reagent, ensure that cell cultures are approximately 3 × 10^6 cells/ml (for suspension cell transfections) at the time of transfection.
Cell morphology has changed	When generating AAV with *Rev*IT™ AAV Enhancer, cell growth may decrease. This is normal and does not adversely affect virus titers.
	Mycoplasma contamination can alter cell morphology and affect transfection efficiency. Check your cells for mycoplasma contamination. Use a fresh frozen stock of cells or use appropriate antibiotics to eliminate mycoplasma.
	A high or low cell passage number can make cells more sensitive and refractory to transfection. Maintain adherent or suspension HEK 293 cells below passage 30 for optimal recombinant virus production.
Transfection complexes not evenly distributed after complex addition to cells	Add transfection complexes while swirling the flask. If this is not possible, gently mix the culture vessel to ensure even distribution of the transfection complexes. However, avoid vigorous agitation that could disturb formed transfection complexes, e.g. vortexing after the initial mixing of the DNA, enhancer and transfection reagent.

RELATED PRODUCTS

- *Trans*IT-VirusGEN® GMP Transfection Reagent
- VirusGEN® AAV Transfection Kit
- VirusGEN® GMP AAV Transfection Kit
- VirusGEN® LV Transfection Kit
- *Rev*IT™ AAV Enhancer GMP
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® Nucleic Acid Labeling Kits
- Ingenio® Electroporation Solution and Kits

Reagent Agent®

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