

## LENTIVIRUS PRODUCTION GUIDELINE

### PART I. INTRODUCTION

Our Lentiviral Packaging Plasmids allows production of replication-deficient, HIV-1-based lentiviruses that can be used to deliver and express your target gene(s) in either dividing or non-dividing mammalian cells. These plasmids supply the helper functions as well as structural and replication proteins in trans required to produce the lentivirus. You will co-transfect the packaging plasmids and the lentiviral expression plasmid containing your target gene into the packaging cell line (e.g., HEK293T) to produce recombinant lentiviruses. The Lentiviral Packaging Plasmids includes the following components:

1. pLP1 Packaging Plasmid encodes the viral core proteins required for forming the structure of the lentivirus and the viral replication enzymes required for replication and integration of the lentivirus.
2. pLP2 Packaging Plasmid encodes the Rev protein that interacts with the RRE on pLP1 to induce Gag and Pol expression.
3. pLP-VSVG Packaging Plasmid encodes the envelope G glycoprotein from Vesicular Stomatitis Virus (VSV-G) to allow production of a pseudotyped lentivirus with a broad host range.
4. pLV-CMV-EGFP-PGK-Puro Control Expression Plasmid contains EGFP reporter gene under the control of CMV promoter and puromycin resistance gene under the PGK promoter. It also contains the elements required to allow packaging of the expression construct into virions (e.g., 5' and 3' LTRs,  $\Psi$  packaging signal). This plasmid can be used to optimize virus production and cell transduction.

### PART II. BIOSAFETY

Our Lentiviral Packaging Plasmids are based on a third-generation system which includes safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus.

1. The number of genes from HIV-1 that are used in the system has been reduced to three (i.e. gag, pol, and rev).
2. The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope.
3. Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids (three packaging plasmids and one expression plasmid). All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus.
4. Although the three packaging plasmids allow expression in trans of proteins required to produce viral progeny (e.g. gag, pol, rev, env) in the packaging cell line, none of them contain LTRs or the  $\Psi$  packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
5. The lentiviruses produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.

6. Expression of the gag and pol genes from pLP1 has been rendered Rev dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev.

Despite the inclusion of the safety features, the lentiviruses produced can still pose some biohazardous risk since they can transduce primary human cells. For this reason, **we highly recommend that you treat the produced lentiviruses as Biosafety Level 2 (BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination.** Furthermore, exercise extra caution when creating lentiviruses carrying potential harmful or toxic genes (e.g. activated oncogenes). For more information about the BL-2 guidelines and lentivirus handling, refer to the document, "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, published by the Centers for Disease Control (CDC). Handle all lentiviruses in compliance with established institutional guidelines. Since safety requirements for use and handling of lentiviruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or officers at your institution.

## PART III. PROTOCOL - LENTIVIRUS PRODUCTION

### 1. General Information

1). Although the plasmids have been designed to help you express your recombinant protein of interest in the simplest, most direct fashion, use of the system is geared towards those users who are familiar with the principles of lentivirus biology and lentiviral vectors. We highly recommend that users possess a working knowledge of virus production and tissue culture techniques.

2). Plasmid DNA for transfection into eukaryotic cells must be very clean and free from contamination with phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. When performing plasmid DNA isolation with commercially available kits from *E. coli* strains (such as Stbl3™) that are wild type for endonuclease 1 (endA1+), ensure that Solution I of the Lysis or Resuspension Buffer contains 10 mM EDTA. EDTA will inactivate the endonuclease and avoid DNA nicking and vector degradation. Alternatively, follow the instructions included the plasmid purification kits for endA1+ *E. coli* strains.

3). The health of your packaging cells at the time of transfection has a critical effect on the success of lentivirus production. Use of "unhealthy" cells will negatively affect the transfection efficiency, resulting in production of a low titer lentiviral stock. For optimal lentivirus production (i.e. producing lentiviral stocks with the expected titers), follow the guidelines below to culture cells before use in transfection:

- Make sure that cells are healthy and greater than 90% viable.
- Do not allow cells to overgrow before passaging.
- Use cells that have been subcultured for less than 10 passages

4). We recommend using our control expression plasmid in your cotransfection experiment to generate a control lentivirus that may be used to help you optimize expression conditions in your mammalian cell line of interest.

### 2. Materials Provided

- pLP1 Packaging Plasmid
- pLP2 Packaging Plasmid
- pLP-VSVG Packaging Plasmid
- pLVX-Puro Expression Plasmid
- pLV-CMV-EGFP-PGK-Puro Control Expression Plasmid

### 3. Materials Needed but Not Provided

- Lentiviral Expression Plasmid containing your gene of interest
- Packaging cell line (e.g., HEK293T)
- Cell culture medium (e.g., Opti-MEM® I Medium)
- Transfection reagent (e.g., Lipofectamine™ 2000)
- Fetal Bovine Serum (FBS)
- Antibiotic Selection Marker (e.g., Puromycin)
- Sterile, 10 cm tissue culture plates
- 15 mL sterile, capped, conical tubes
- Cryovials

### 4. Lentivirus Production

We recommend including a negative control (no DNA, no transfection reagent) in your experiment to help you evaluate your results. We produce lentiviruses in HEK293T cells using the following optimized transfection conditions below.

Condition	Quantity
Tissue culture plate size	10 cm (one per lentiviral construct)
Number of HEK293T cells to transfect	$6 \times 10^6$ cells
pLV-CMV-EGFP-PGK-Puro Control Expression Plasmid	3 $\mu$ g
pLP1 Packaging Plasmid	3.34 $\mu$ g
pLP2 Packaging Plasmid	2.21 $\mu$ g
pLP-VSVG Packaging Plasmid	3.34 $\mu$ g
Amount of Lipofectamine™ 2000	36 $\mu$ L

**Note:** You may produce lentiviral stocks using other tissue culture formats, but keep in mind that optimization will be necessary to obtain the expected titers.

- 1). The day before transfection (Day 1), plate HEK293T cells in a 10 cm tissue culture plate so that they will be 90~95% confluent on the day of transfection (i.e.  $5 \times 10^6$  cells in 10 mL of growth medium containing serum). Do not include antibiotics in the medium.
- 2). On the day of transfection (Day 2), remove the culture medium from the HEK293T cells and replace with 5 mL of growth medium containing serum. Do not include antibiotics in the medium.
- 3). For each transfection sample, prepare DNA-Lipofectamine™ 2000 complexes as follows:
  - 3-1). In a sterile 5 mL tube, dilute 9  $\mu$ g of the Packaging Plasmids (3.34  $\mu$ g pLP1, 2.21  $\mu$ g pLP2 and 3.34  $\mu$ g pLP/VSV-G) and 3  $\mu$ g of pLenti expression plasmid DNA in 1.5 mL of Opti-MEM® I Medium without serum. Mix gently.

3-2). In a separate sterile 5 mL tube, mix Lipofectamine™ 2000 gently before use, then dilute 36 µL in 1.5 mL of Opti-MEM® I Medium without serum. Mix gently and incubate for 5 minutes at room temperature.

3-3). After the 5 minute incubation, combine the diluted DNA with the diluted Lipofectamine™ 2000. Mix gently.

3-4). Incubate for 20 minutes at room temperature to allow the DNA-Lipofectamine™ 2000 complexes to form. The solution may appear cloudy, but this will not impede the transfection.

4). Add the DNA-Lipofectamine™ 2000 complexes dropwise to each plate of cells. Mix gently by rocking the plate back and forth. Incubate the cells overnight at 37°C in a humidified 5% CO<sub>2</sub> incubator.

5). The next day (Day 3), remove the medium containing the DNA-Lipofectamine™ 2000 complexes and replace with 10 mL complete culture medium without antibiotics. Incubate at 37°C in a humidified 5% CO<sub>2</sub> incubator.

**Note:** *Expression of the VSV G glycoprotein causes HEK293T cells to fuse, resulting in the appearance of large, multinucleated cells known as syncytia. This morphological change is normal and does not affect production of the lentivirus.*

6). Harvest virus-containing supernatants 48~72 hours posttransfection (Day 4~5) by removing medium into to a 15 mL sterile, capped, conical tube. Minimal differences in viral yield are observed whether supernatants are collected at either 48 or 72 hours posttransfection.

**Caution:** *Remember that you are working with infectious virus at this stage. Follow the recommended guidelines for working with BL-2 organisms.*

7). Centrifuge supernatants at 3000 rpm for 15 minutes at 4°C to pellet debris.

8). **Optional:** If you plan to use your lentiviral construct for in vivo applications, we recommend filtering your viral supernatant through a sterile, 0.45 µm low protein binding filter after the low-speed centrifugation step to remove any remaining cellular debris. We recommend using Millex-HV 0.45 µm PVDF filters for filtration.

9). If you wish to concentrate your viral stock to obtain a higher titer, perform the filtration step first before concentrating your viral stock. It is possible to concentrate VSV-G pseudotyped lentiviruses using a variety of methods without significantly affecting their ability to transduce cells. If your cell transduction experiment requires that you use a relatively high MOI, you may wish to concentrate your virus before titering and proceeding to transduction. For details and guidelines to concentrate your virus supernatant by ultracentrifugation, refer to published reference sources (Yee, 1999, The Development of Human Gene Therapy). You could also use the commercial kit to concentrate and purify your lentivirus.

10). Pipet viral supernatants into cryovials in appropriated aliquots. Store viral stocks at -80°C for long-term storage. Repeated freezing and thawing is not recommended as it may result in loss of viral titer. When stored properly, viral stocks of an appropriate titer should be suitable for use for up to one year. After long-term storage, we recommend retitering your viral stocks before transducing your mammalian cell line of interest.

11). It is possible to scale up the cotransfection experiment to produce a larger volume of lentivirus, if desired. If you wish to scale up your cotransfection, remember that you will need to increase the number of cells plated and the amounts of DNA, transfection reagent, and medium used in proportion to the difference in surface area of the culture vessel.

#### PART IV. PROTOCOL - LENTIVIRUS TITRATION

## 1. General Information

1). A number of factors can influence viral titers including:

- The size of your gene of interest. Titters will decrease as the size of the insert increases.
- The characteristics of the cell line used for titering- We strongly recommend the human fibrosarcoma line HT1080 as the “gold standard” for reproducibly titering lentivirus. However, other cell lines (e.g., HEK293) may be used. In general, these cells should be an adherent, non-migratory cell line, and exhibit a doubling time in the range of 18-25 hours.
- Viral titers may decrease with long-term (>1 year) storage at -80°C. If your lentiviral stock has been stored for longer than 6 months, we recommend titering your lentiviral stock prior to use.
- Viral titers can decrease as much as 10% with each freeze/thaw cycle.
- Improper storage of your lentiviral stock- Lentiviral stocks should be stored at -80°C in cryovials.

2). You need to determine the minimum concentration of the antibiotic marker required to kill your untransduced mammalian cell line (i.e. perform a kill curve experiment). We recommend that you test a range of concentrations (see below)

- Plate cells at approximately 25% confluence. Prepare a set of 6~7 plates. Allow cells to adhere overnight.
- The next day, substitute culture medium with medium containing varying concentrations of the antibiotic marker, as appropriate.
- Replenish the selective media every 3~4 days, and observe the percentage of surviving cells.
- Determine the appropriate concentration of the antibiotic marker that kills the cells within 10~14 days after addition of antibiotic.

3). Lentivirus transduction may be enhanced if cells are transduced in the presence of hexadimethrine bromide (Polybrene®). For best results, we recommend performing transduction in the presence of Polybrene®. Note however, that some cells are sensitive to Polybrene® (e.g. primary neurons). Before performing any transduction experiments, you may want to test your cell line for sensitivity to Polybrene® at a range of 0-10 µg/mL. If your cells are sensitive to Polybrene® (e.g. exhibit toxicity or phenotypic changes), do not add Polybrene® during transduction. In this case, cells should still be successfully transduced with your lentivirus.

## 2. Transduction and Titering Procedure

Follow the procedure below to determine the titer of your lentiviral stock using the mammalian cell line of choice. You will use at least one 6-well plate for every lentiviral stock to be titered (one mock well plus five dilutions).

1). The day before transduction (Day 1), trypsinize and count the cells, plating them in a 6-well plate such that they will be 30~50% confluent at the time of transduction. Incubate cells at 37°C overnight in a humidified 5% CO<sub>2</sub> incubator. Example: When using HT1080 cells, we usually plate  $2 \times 10^5$  cells per well in a 6-well plate.

2). On the day of transduction (Day 2), thaw your lentiviral stock and prepare 10-fold serial dilutions ranging from  $10^{-2}$  to  $10^{-6}$ . For each dilution, dilute the lentiviral stock into complete culture medium to a final volume of 1 mL. DO NOT vortex.

**Note:** You may prepare a wider range of serial dilutions ( $10^{-2}$  to  $10^{-8}$ ), if desired.

3). Remove the culture medium from the cells. Mix each dilution gently by inversion and add to one well of

cells (total volume = 1 mL).

- 4). Add Polybrene® (if desired) to each well to a final concentration of 6 µg/mL. Swirl the plate gently to mix. Incubate at 37°C overnight in a humidified 5% CO<sub>2</sub> incubator.
- 5). The following day (Day 3), remove the media containing virus and replace with 2 mL of complete culture medium. Incubate at 37°C overnight in a humidified 5% CO<sub>2</sub> incubator.
- 6). The following day (Day 4), remove the medium and replace with complete culture medium containing the appropriate amount of the antibiotic marker to select for stably transduced cells.
- 7). Replace medium with fresh medium containing antibiotic every 3~4 days.
- 8). After 10~12 days of selection (day 14~16), you should see no live cells in the mock well and discrete antibiotic-resistant colonies in one or more of the dilution wells. Remove the medium and wash the cells twice with PBS.
- 9). Add crystal violet solution (1 mL for 6-well dish; 5 mL for 10 cm plate) and incubate for 10 minutes at room temperature.
- 10). Remove the crystal violet stain and wash the cells with PBS. Repeat wash.
- 11). Count the blue-stained colonies and determine the titer of your lentiviral stock.

For example, if the colony counts were:

- Mock: no colonies
- 10<sup>2</sup> dilution: confluent; undeterminable
- 10<sup>3</sup> dilution: confluent; undeterminable
- 10<sup>4</sup> dilution: confluent; undeterminable
- 10<sup>5</sup> dilution: 46
- 10<sup>6</sup> dilution: 5

Thus, the titer of this lentiviral stock is  $4.8 \times 10^6$  TU/ml (average of  $46 \times 10^5$  and  $5 \times 10^6$ ).

### 3. Real-Time PCR-Based Assay for Lentiviral Titration

Lentivirus titer can also be determined through quantitative PCR (qPCR) assay by measuring the number of copies of lentiviruses stably integrated into the genome after transduction. For detailed assay procedure, please refer to the published paper (Barczak W, Suchorska W, Rubiś B, Kulcenty K. Universal real-time PCR-based assay for lentiviral titration. Mol Biotechnol. 2015 Feb;57(2):195-200. doi: 10.1007/s12033-014-9815-4. PMID: 25370825; PMCID: PMC4298670.).

## PART V. PROTOCOL - TRANSDUCTION AND ANALYSIS

### 1. General Information

1). Once you have generated a lentiviral stock with a suitable titer, you are ready to transduce the lentiviral construct into the mammalian cell line of choice and assay for expression of your recombinant protein. After transducing your lentiviral construct into the mammalian cell line of choice, you may assay for expression of your gene of interest in the following ways:

- Pool a heterogeneous population of cells and test for expression directly after transduction (i.e. “transient” expression). Note that you must wait for a minimum of 48~72 hours after transduction before harvesting your cells to allow expressed protein to accumulate in transduced cells.

- Select for stably transduced cells using antibiotic selection. This requires a minimum of 10-12 days after transduction, but allows generation of clonal cell lines that stably express the gene of interest.
- 2). If you wish to select for stably transduced cells, you must first determine the minimum concentration of the antibiotic marker, as appropriate, required to kill your untransduced mammalian cell line (i.e. perform a kill curve experiment).
- 3). To obtain optimal expression of your gene of interest, you will need to transduce the lentiviral construct into your mammalian cell line of choice using a suitable MOI. MOI is defined as the number of virus particles per cell and generally correlates with the number of integration events and as a result, expression of your gene of interest. Typically, expression levels increase linearly as the MOI increases. A number of factors can influence optimal MOI including the nature of your mammalian cell line (e.g. non-dividing vs. dividing cell type), its transduction efficiency, your application of interest, and the nature of your gene of interest. If you are transducing your lentiviral construct into the mammalian cell line of choice for the first time, we recommend using a range of MOI (e.g. 0, 0.5, 1, 2, 5, 10 etc.) to determine the MOI required to obtain the optimal expression of your protein for your application.
- 4). We recommend using the EGFP control lentivirus to help you determine the optimal MOI for your particular cell line and application. Once you have transduced the control lentivirus into your mammalian cell line of choice, the EGFP will be constitutively expressed and can be easily assayed.
- 5). Viral supernatants are generated by harvesting spent media containing virus from the HEK293T producer cells. Spent media lacks nutrients and may contain some toxic metabolic waste products. If you are using a large volume of viral supernatant to transduce your mammalian cell line, note that growth characteristics or morphology of the cells may be affected during transduction. These effects are generally alleviated after transduction when the media is replaced with fresh, complete media.

## 2. Transduction Procedure

Follow the procedure below to transduce the mammalian cell line of choice with your lentiviral construct.

- 1). Plate cells in complete media as appropriate for your application.
- 2). On the day of transduction (Day 1), thaw your lentiviral stock, and if necessary, dilute the appropriate amount of virus into fresh complete medium to obtain a suitable MOI. Keep the total volume of medium containing virus as low as possible to maximize transduction efficiency. DO NOT vortex.
- 3). Remove the culture medium from the cells. Mix the medium containing virus gently by pipetting and add to the cells.
- 4). Add Polybrene® (if desired) to a final concentration up to 8 µg/ml. Swirl the plate gently to mix. Incubate at 37°C in a humidified 5% CO<sub>2</sub> incubator overnight.  
**Note:** *If you are transducing cells with undiluted viral stock and are concerned about possible toxicity or growth effects caused by overnight incubation, it is possible to incubate cells for as little as 6 hours prior to changing medium.*
- 5). The following day (Day 2), remove the medium containing virus and replace with fresh, complete culture medium. Incubate at 37°C in a humidified 5% CO<sub>2</sub> incubator overnight.
- 6). The following day (Day 3), perform one of the following:
  - Harvest the cells and assay for expression of your recombinant protein if you are performing transient expression experiments.
  - Remove the medium and replace with fresh, complete medium containing the appropriate amount of the

antibiotic marker , as appropriate to select for stably transduced cells.

7). Replace medium with fresh medium containing antibiotic every 3-4 days until antibiotic-resistant colonies can be identified (generally 10~12 days after selection).

8). Pick at least 5 antibiotic-resistant colonies and expand each clone to assay for expression of the recombinant protein.

**Note:** *Integration of the lentivirus into the genome is random. Depending upon the influence of the surrounding genomic sequences at the integration site, you may see varying levels of recombinant protein expression from different antibiotic-resistant clones. We recommend testing at least 5 antibiotic-resistant clones and selecting the clone that provides the optimal expression of your recombinant protein for further studies.*

## PART VI. TROUBLESHOOTING

The table below lists some potential problems and possible solutions that may help you troubleshoot your cotransfection, titrating, transduction and expression experiments.

### 1. Lentivirus Production

<b>Problem 1: Low virus titer</b>	
<b>Cause</b>	<b>Solution</b>
Low transfection efficiency: <ul style="list-style-type: none"> <li>• Used poor quality expression construct plasmid DNA (i.e. plasmid DNA from a mini-prep)</li> <li>• Unhealthy HEK293T cells; cells exhibit low viability</li> <li>• Cells transfected in media containing antibiotics</li> <li>• Plasmid DNA: transfection reagent ratio incorrect</li> <li>• Insufficient co-transfection</li> <li>• HEK293T cells plated too sparsely</li> </ul>	<ul style="list-style-type: none"> <li>• Do not use mini-prep plasmid DNA for transfection. Use Midiprep DNA Isolation Kit to prepare plasmid DNA.</li> <li>• Use healthy HEK293T cells under passage 10; do not overgrow.</li> <li>• Do not add antibiotics to media during transfection as this reduces transfection efficiency and causes cell death.</li> <li>• Use a DNA (in µg):Lipofectamine™ 2000 (in µL) ratio ranging from 1:2 to 1:3.</li> <li>• Use more DNA/ Lipofectamine™ 2000 (keeping the ratios the same).</li> <li>• Plate cells such that they are 90~95% confluent at the time of transfection.</li> </ul>
Transfected cells not cultured in media containing sodium pyruvate	One day after transfection, remove media containing DNA-lipid complexes and replace with media containing sodium pyruvate. Sodium pyruvate provides an extra energy source for the cells.
Viral supernatant harvested too early	Viral supernatants can generally be collected 48~72 hours post-transfection. If many cells are still attached to the plate and look healthy at this point, wait an additional 24 hours before harvesting the viral supernatant.
Viral supernatant too dilute	Concentrate your virus.



Viral supernatant frozen and thawed multiple times	Do not freeze/thaw viral supernatant more than 3 times.
Poor choice of titering cell line	Use HT1080 cells or another adherent cell line.
Gene of interest is toxic to cells	Do not generate constructs containing activated oncogenes or harmful genes.
Gene of interest is large	Viral titers generally decrease as the size of the insert increases. Concentrate the virus if titer is low.
Polybrene® not included during transduction	Transduce the lentiviral construct into cells in the presence of Polybrene®.
Lipofectamine™ 2000 handled incorrectly	<ul style="list-style-type: none"> <li>• Store at 4°C. Do not freeze.</li> <li>• Mix gently by inversion. Do not vortex.</li> </ul>
<b>Problem 2: No colonies obtained upon titering</b>	
<b>Cause</b>	<b>Solution</b>
Too much antibiotic used for selection	Determine the antibiotic sensitivity of your cell line by performing a kill curve experiment, and use the minimum concentration required to kill your untransduced cell line.
Viral stocks stored incorrectly	Aliquot and store stocks at -80°C. Do not freeze/thaw more than 3 times.
Polybrene® not included during transduction	Transduce the lentiviral construct into cells in the presence of Polybrene®.
<b>Problem 3: Titer indeterminable; cells confluent</b>	
<b>Cause</b>	<b>Solution</b>
Too little antibiotic used for selection	Increase amount of antibiotic.
Antibiotic selection performed on confluent cells	Before adding selective medium, trypsinize transduced cells and replat in a larger tissue culture plate.
Viral supernatant not diluted sufficiently	Titer lentivirus using a wider range of 10-fold serial dilutions (e.g. 10 <sup>-2</sup> to 10 <sup>-8</sup> ).

## 2. Mammalian Cell Transduction

<b>Problem 1: No expression of the gene of interest</b>	
<b>Cause</b>	<b>Solution</b>
Promoter silencing	<ul style="list-style-type: none"> <li>• Lentiviral constructs may integrate into a chromosomal region that silences the CMV promoter. Screen multiple antibiotic resistant clones and select the one with the highest expression levels.</li> </ul>
Viral stocks stored incorrectly	Aliquot and store stocks at -80°C. Do not freeze/thaw more than 3 times.
<b>Problem 2: Poor expression of the gene of interest</b>	
<b>Cause</b>	<b>Solution</b>

Low transduction efficiency: <ul style="list-style-type: none"> <li>• Polybrene® not included during transduction</li> <li>• Non-dividing cell type used</li> </ul>	<ul style="list-style-type: none"> <li>• Transduce the lentiviral construct into cells in the presence of Polybrene®.</li> <li>• Transduce your lentiviral construct into cells using a higher MOI.</li> </ul>
MOI too low	Transduce your lentiviral construct into cells using a higher MOI.
Too much antibiotic used for selection	Determine the antibiotic sensitivity of your cell line by performing a kill curve. Use the minimum antibiotic concentration required to kill your untransduced cell line.
Cells harvested too soon after transduction	Do not harvest cells until at least 48~72 hours after transduction to allow expressed protein to accumulate in transduced cells.
Gene of interest is toxic to cells	Generating constructs containing activated oncogenes or potentially harmful genes is not recommended.
<b>Problem 3: Cytotoxic effects observed after transduction</b>	
<b>Cause</b>	<b>Solution</b>
Large volume of viral supernatant used for transduction	<ul style="list-style-type: none"> <li>• Remove the “spent” media containing virus and replace with fresh, complete media.</li> <li>• Concentrate the virus (Yee, 1999).</li> </ul>
Polybrene® used during transduction	Verify the sensitivity of your cells to Polybrene®. If cells are sensitive, omit the Polybrene® during transduction.
Too much antibiotic used for selection	Determine the antibiotic sensitivity of your cell line by performing a kill curve. Use the minimum concentration of antibiotic required to kill your untransduced cell line.
Gene of interest is toxic to cells	Try a different cell line.

## PART VII. STATEMENT

The Products provided by RGBiotech shall be used by the purchaser for internal research purposes only unless otherwise indicated. Use of this product by a purchaser for any purpose other than for research, including, but not limited to, in vitro diagnostic purposes, therapeutics, or in humans, is unauthorized and prohibited. RGBiotech disclaims the responsibility for any damage or injury that may be caused by the failure of the purchaser or any other person to follow the guidelines sent out by the NIH guidelines for recombinant DNA and Gene transfer. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.