invitrogen™
by life technologies™

ViraPower™ HiPerform™ Lentiviral Expression Systems

Lentiviral systems for high-level expression in dividing and non-dividing mammalian cells

Cat. nos. K5310-00, K5320-00, K5330-00 and K5340-00

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User Manual

Contents

Kit Contents and Storage

Types of Kits

This manual is supplied with the kits listed below. The ViraPower™ HiPerform™ Lentiviral Expression Kits include the ViraPower™ HiPerform™ Lentiviral Support Kit, an expression vector, and the 293FT producer cell line. The ViraPower™ Lentiviral Support Kits include the ViraPower™ Packaging Mix, Lipofectamine® 2000, and a selection agent.

Product	Cat. no.
ViraPower™ HiPerform™ Lentiviral TOPO® Expression Kit	K5310-00
ViraPower™ HiPerform™ Lentiviral FastTiter™ TOPO® Expression Kit	K5320-00
ViraPower™ HiPerform™ Lentiviral Gateway® Expression Kit	K5330-00
ViraPower™ HiPerform™ Lentiviral FastTiter™ Gateway® Expression Kit	K5340-00

Intended Use

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

System Components

The following table shows the components associated with ViraPower™ HiPerform™ Lentiviral Expression Kits. For detailed instructions to grow and maintain the 293FT Cell Line, see the 293FT Cell Line manual.

Components	Cat. no.			
	K5310-00	K5320-00	K5330-00	K5340-00
pLenti6.3/V5-TOPO® TA Cloning® Kit	✓			
pLenti7.3/V5-TOPO® TA Cloning® Kit		✓		
pLenti6.3/V5-DEST™ Gateway® Vector Kit			✓	
pLenti7.3/V5-DEST™ Gateway® Vector Kit				✓
ViraPower™ Lentiviral Support Kit	✓	✓	✓	✓
One Shot® Stbl3™ Chemically Competent *E. coli*	✓	✓	✓	✓
293FT Cell Line	✓	✓	✓	✓
Blasticidin	✓		✓	

Continued on next page

Kit Contents and Storage, Continued

Shipping and Storage

The ViraPower™ HiPerform™ Lentiviral products are shipped as described below. Upon receipt, store each component as detailed below.

Item	Shipping	Storage
293FT Cell Line	Dry ice	Liquid nitrogen
Blasticidin	Room temperature	–20°C
ViraPower™ Packaging Mix	Room temperature	–20°C
ViraPower™ Lentiviral Support Kit: • ViraPower™ Packaging Mix • Lipofectamine® 2000	Blue ice	 –20°C 4°C **(do not freeze)**
pLenti6.3/V5-TOPO® TA Cloning® Kit: • Vectors • One Shot® Stbl3™ Chemically Competent *E. coli*	Dry ice	 –20°C –80°C
pLenti7.3/V5-TOPO® TA Cloning® Kit: • Vectors • One Shot® Stbl3™ Chemically Competent *E. coli*	Dry ice	 –20°C –80°C
pLenti6.3/V5-DEST™ Gateway® Vector Kit: • Vectors • One Shot® Stbl3™ Chemically Competent *E. coli*	Dry ice	 –20°C –80°C
pLenti7.3/V5-DEST™ Gateway® Vector Kit: • Vectors • One Shot® Stbl3™ Chemically Competent *E. coli*	Dry ice	 –20°C –80°C

Expression Vectors

Each ViraPower™ HiPerform™ Lentiviral Expression Kit also includes a pLenti-based expression vector kit. The expression vector kit includes:

- A pLenti-based expression vector for cloning your gene of interest
- A corresponding expression control plasmid
- One Shot® Stbl3™ Chemically Competent *E. coli* for transformation

Each vector manual supplied with the kit contains a detailed description of the provided reagents and instructions to generate an expression vector with your gene of interest.

Vector	Cat. no.
pLenti6.3/V5-TOPO® vector	K5310-00
pLenti7.3/V5-TOPO® vector	K5320-00
pLenti6.3/V5-DEST™ Gateway vector	K5330-00
pLenti7.3/V5-DEST™ Gateway vector	K5340-00

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Kit Contents and Storage, Continued

ViraPower™ Lentiviral Support Kit Contents

The ViraPower™ HiPerform™ Lentiviral Support Kit includes the following vectors and reagents. Store as directed below. Additional packaging mix (ViraPower™ Lentiviral Packaging Mix) and Lipofectamine® 2000 Reagent may be purchased separately; see page 44 for ordering information.

Important: Do not freeze Lipofectamine® 2000 Reagent.

Reagent	Composition	Amount	Storage
ViraPower™ Packaging Mix	Contains a mixture of the pLP1, pLP2, and pLP/VSVG plasmids, 1 µg/µL in TE, pH 8.0	195 µg	–20°C
Lipofectamine® 2000	Proprietary	0.75 mL	4°C

One Shot® Stbl3™ Chemically Competent *E. coli*

The following reagents are included with the One Shot® Stbl3™ Chemically Competent *E. coli* kit. Transformation efficiency is ≥ 1 × 10^8 cfu/µg plasmid DNA. **Store at –80°C.**

Reagent	Composition	Amount
S.O.C. Medium	2% Tryptone 0.5% Yeast Extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	6 mL
Stbl3™ Cells	—	21 × 50 µL
pUC19 Control DNA	10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8.0	50 µL

Genotype of Stbl3™ Cells

F$^-$ *mcr*B *mrr hsd*S20(r_B^-, m_B^-) *rec*A13 *sup*E44 *ara*-14 *gal*K2 *lac*Y1 *pro*A2 *rps*L20(Str^R) *xyl*-5 λ^- *leu mtl*-1

Note: This strain is *end*A1+

293FT Cell Line

Each ViraPower™ HiPerform™ Lentiviral Expression Kit includes the 293FT producer cell line. The 293FT Cell Line is supplied as one vial containing 3 × 10^6 frozen cells in 1 mL of Freezing Medium. **Upon receipt, store in liquid nitrogen.**

For instructions on how to thaw, culture, and maintain the 293FT Cell Line, see the 293FT Cell Line manual, included with the ViraPower™ HiPerform™ Lentiviral Expression Kit. To download the manual, visit www.invitrogen.com, or contact **Technical Support** (page 45).

Introduction

System Summary

Description of the System

The new ViraPower™ HiPerform™ Lentiviral Expression Systems allow the creation of a replication-incompetent, HIV-1-based lentivirus to deliver and express a gene of interest in dividing or non-dividing mammalian cells. The new expression systems use four new expression vectors:

- pLenti6.3/V5-DEST™ Gateway® vector and pLenti7.3/V5-DEST™ Gateway vectors, which are pLenti Gateway Destination vectors adapted for use with the Gateway® technology
- pLenti6.3/V5-TOPO® vector and pLenti7.3/V5-TOPO® vectors, which are pLenti TOPO® vectors that combine the ViraPower™ HiPerform™ Lentiviral Expression Systems with the rapid TOPO® Cloning technology

ViraPower™ HiPerform™ Lentiviral Expression Vectors

The new ViraPower™ HiPerform™ Lentiviral Expression vectors contain two new elements (WPRE and cPPT) to yield cell-specific, high-performance results. The Woodchuck Posttranscriptional Regulatory Element (**WPRE**) from the woodchuck hepatitis virus, placed directly downstream of the gene of interest, allows for increased transgene expression (Zufferey *et al.*, 1998), with more cells expressing the gene of interest. **cPPT** (Polypurine Tract) from the HIV-1 integrase gene increases the copy number of lentivirus integrating into the host genome (Park, 2001) and allows for a two-fold increase in viral titer. Together, WPRE and cPPT produce at least a four-fold protein expression increase in most cell types compared to other vectors that do not contain these elements. The ViraPower™ HiPerform™ Lentiviral **FastTiter**™ Expression Systems (Cat. nos. K5320-00 and K5340-00) allow for an accurate titer of functional lentivirus in just **two days** using EmGFP.

The ViraPower™ HiPerform™ Lentiviral Expression System vectors also contain all of the following:

- Human cytomegalovirus (CMV) immediate early promoter to control high-level expression of the gene of interest in all four vectors.
- C-terminal V5 tag for convenient detection.
- SV40 promoter driving the expression of Blasticidin (pLenti6.3/V5-DEST™ Gateway® vector and pLenti6.3/V5-TOPO® Vector), **or** Emerald Green Fluorescent Protein (EmGFP, derived from *Aequorea Victoria* GFP, pLenti7.3/V5-DEST™ Gateway® and pLenti7.3/V5-TOPO® vector).
- Blasticidin resistance gene (Izumi *et al.*, 1991; Kimura *et al.*, 1994; Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965) for stable transduction and selection in *E. coli* and mammalian cells (pLenti6.3/V5-DEST™ Gateway® and pLenti6.3/V5-TOPO® TA vectors, only) **or** EmGFP (pLenti7.3/V5-DEST™ Gateway® and pLenti7.3/V5-TOPO® vectors only) for easy determination of the Lentiviral titer by flow cytometry.

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Components of the ViraPower™ HiPerform™ Lentiviral Expression System

The ViraPower™ HiPerform™ Lentiviral Expression Systems facilitate highly efficient *in vitro* delivery of a target gene to dividing and non-dividing mammalian cells using a replication-incompetent lentivirus. Based on the lentikat™ system developed by Cell Genesys (Dull *et al.*, 1998), the ViraPower™ HiPerform™ Lentiviral Expression System possesses features that enhance its biosafety while allowing high-level gene expression in a wider range of cell types than traditional retroviral systems. The System includes the following major components:

- A pLenti-based expression vector into which the gene of interest is cloned. The vector contains the WPRE and cPPT elements, which allows more cells to express the gene of interest at higher levels and faster titering times. The vector also contains the elements required for packaging the expression construct into virions (e.g., 5′ and 3′ LTRs, Ψ packaging signal). For more information about the pLenti expression vectors, refer to the manual for the specific vector you are using.
- The ViraPower™ Packaging Mix containing an optimized mixture of the three packaging plasmids, pLP1, pLP2, and pLP/VSVG. These plasmids supply the helper functions and the structural and replication proteins *in trans* required to produce the lentivirus. The pLP/VSVG plasmid in the packaging mix contains the G glycoprotein gene from Vesicular Stomatitis Virus (VSV-G) as a pseudotyping envelope, which allows the production of a high titer lentiviral vector with a significantly broadened host cell range (Burns *et al.*, 1993; Emi *et al.*, 1991; Yee *et al.*, 1994). For more information about the packaging plasmids, see the **Appendix**, pages 38–43.
- An optimized 293FT producer cell line that stably expresses the SV40 large T-antigen under the control of the human CMV promoter and facilitates optimal production of virus. For more information about the 293FT Cell Line, refer to the 293FT Cell Line manual.

You will cotransfect the ViraPower™ Packaging Mix and the pLenti vector containing the gene of interest into 293FT cells to produce a replication-incompetent lentivirus, and then use this lentivirus to transduce a mammalian cell line of interest.

Continued on next page

System Summary, Continued

Features of the ViraPower™ HiPerform™ Lentiviral Systems

The major features of the ViraPower™ HiPerform™ Lentiviral Systems include:

- An expression plasmid containing the gene of interest under the control of a CMV early promoter, and elements that allow packaging of the construct into virions.
- Polypurine Tract from HIV (cPPT) for increased viral titer (Park *et al.*, 2001).
- WPRE for increased transgene expression (Zufferey *et al.*, 1999).
- An optimized mix of the three packaging plasmids (pLP1, pLP2, and pLP/VSVG) that supply the structural and replication proteins *in trans* that are required to produce the lentivirus.
- The 293FT cell line, which allows the production of lentivirus following cotransfection of the expression plasmid and the plasmids in the packaging mix.
- Control expression plasmid to optimize virus production and cell transduction, containing either:
    - The *lacZ* gene, which when packaged into virions and transduced into a mammalian cell line, expresses β-galactosidase (included with each expression vector kit)

    or

    - The Emerald Green Fluorescent Protein (EmGFP) gene, which expresses EmGFP when packaged into virions and transduced into a mammalian cell line (available separately, see page 44).

For more information on expression vectors and the corresponding positive control vectors, refer to the manual for the specific expression or control vector you are using.

Continued on next page

System Summary, Continued

Advantages of the System

Using the ViraPower™ HiPerform™ Lentiviral Expression System to facilitate lentiviral-based expression of the gene of interest provides the following advantages:

- Offers you a choice to use Gateway® technology (Cat. nos. K5330-00 and K5340-00) or TOPO® Cloning technologies (Cat. nos. K5310-00 and K5320-00).
- Promotes enhanced protein expression, up to 4-fold or greater.
- Generates an HIV-1-based lentivirus that effectively transduces dividing and non-dividing mammalian cells, thus broadening the potential applications beyond those of traditional Moloney Murine Leukemia Virus (MoMLV)-based retroviral systems (Naldini, 1998).
- Efficiently delivers the gene of interest to mammalian cells in culture or *in vivo* (Dull *et al*., 1998).
- Provides stable, long-term expression of a target gene beyond that offered by traditional adenoviral-based systems (Dull *et al*., 1998; Naldini *et al*., 1996).
- Produces a pseudotyped virus with a broadened host range (Yee *et al*., 1994).
- Includes multiple features designed to enhance the biosafety of the system.
- pLenti6.3 series vectors offer significantly improved levels of protein expression by increasing the number of cells that express the cloned gene of interest.
- pLenti7.3 series vectors offer significantly improved levels of expression of your gene of interest. pLenti7.3 vectors also allow high-speed and high-throughput titering applications using EmGFP, and reduce the titering time to **2 days**.

How Lentivirus Works

When the lentivirus enters the target cell, the viral RNA is reverse-transcribed, actively imported into the nucleus (Lewis & Emerman, 1994; Naldini, 1999), and stably integrated into the host genome (Buchschacher & Wong-Staal, 2000; Luciw, 1996). After the lentiviral construct has integrated into the genome, you may assay for transient expression of your recombinant protein or use antibiotic selection to generate a stable cell line for long-term expression studies.

Continued on next page

System Summary, Continued

Purpose of this Manual

This manual presents an overview of the ViraPower™ HiPerform™ Lentiviral Expression System and provides instructions and guidelines to:

1. Co-transfect the pLenti-based expression vector and the ViraPower™ Packaging Mix into the 293FT Cell Line to produce a lentiviral stock.
2. Titer the lentiviral stock.
3. Use the lentiviral stock to transduce a mammalian cell line of choice.
4. Assay for "transient" expression of your recombinant protein,

   or

   Generate a stably transduced cell line.

For details and instructions to generate your expression vector, refer to the manual for the pLenti vector you are using. For instructions to culture and maintain the 293FT producer cell line, refer to the 293FT Cell Line manual. These manuals are supplied with the ViraPower™ HiPerform™ Lentiviral Expression Kits, and are also available at www.invitrogen.com or by contacting **Technical Support** (page 45).

Biosafety Features of the System

Introduction

The ViraPower™ HiPerform™ Lentiviral Expression System is a fourth-generation lentiviral vector-based system developed by Dull *et al*., 1998. It includes a significant number of safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus. These safety features are discussed below.

Biosafety Features of the ViraPower™ HiPerform™ Lentiviral System

The ViraPower™ HiPerform™ Lentiviral Expression System includes the following key safety features:

- The pLenti expression vector contains a deletion in the 3′ LTR (ΔU3) that does not affect the generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee *et al*., 1987; Yu *et al*., 1986; Zufferey *et al*., 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
- The number of genes from HIV-1 used in the system has been reduced to three (i.e. *gag*, *pol*, and *rev*).
- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns *et al*., 1993; Emi *et al*., 1991; Yee *et al*., 1994).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. 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 (Dull *et al*., 1998).
- Although the three packaging plasmids allow *in trans* expression of proteins required to produce viral progeny (e.g., gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ 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.
- The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.
- 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 (Dull *et al*., 1998).
- A constitutive promoter (RSV promoter) has been placed upstream of the 5′ LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA (Dull *et al*., 1998).

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Biosafety Features of the System, Continued

Biosafety Level 2

Despite the inclusion of the safety features discussed on the previous page, the lentivirus produced with this system can still pose some biohazardous risk because it can transduce primary human cells. For this reason, **we highly recommend that you treat lentiviral stocks generated using this system as Biosafety Level 2 (BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination.** Exercise extra caution when creating lentivirus that carry 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," 5th Edition, published by the Centers for Disease Control (CDC). This document is available at:

www.cdc.gov/biosafety/publications/index.htm

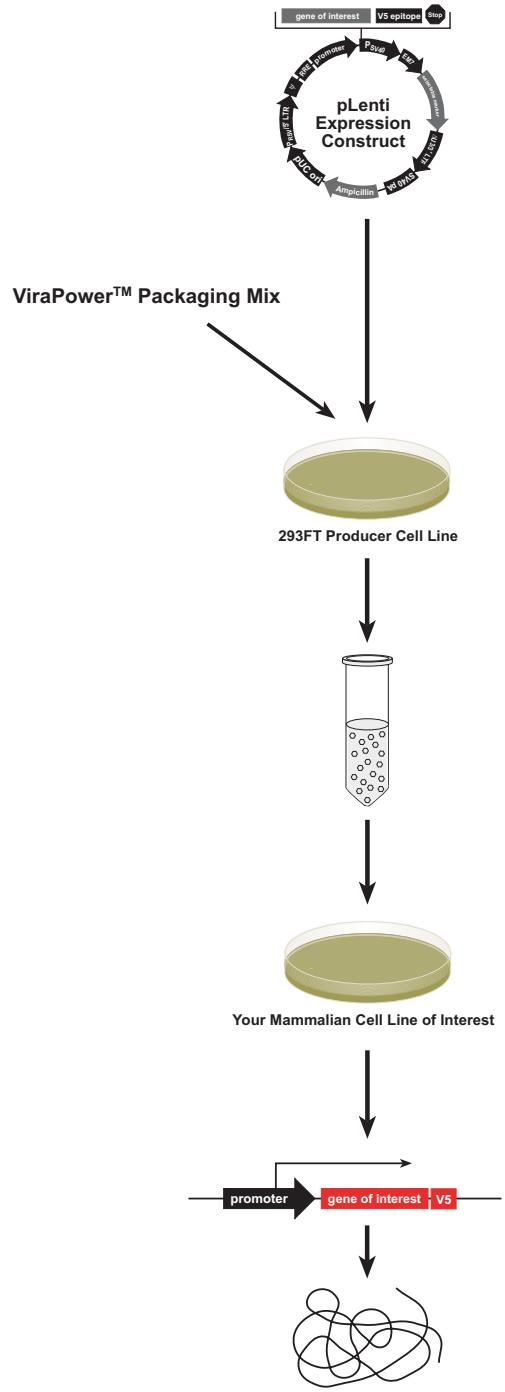
Important

Handle all lentiviruses in compliance with established institutional guidelines. Because safety requirements for use and handling of lentiviruses may vary at individual institutions, consult the health and safety guidelines and/or officers at your institution before using the ViraPower™ HiPerform™ Lentiviral Expression System.

Experimental Outline

Flow Chart

The diagram below describes the general steps required to express your gene of interest using the ViraPower™ HiPerform™ Lentiviral Expression System. Refer to the appropriate manual for each pLenti expression vector for instructions to generate your pLenti expression construct.



1. Generate the pLenti expression construct containing your gene of interest.
2. Cotransfect the 293FT producer cell line with your pLenti expression construct and the optimized packaging mix.
3. Harvest viral supernatant and determine the titer.
4. Add the viral supernatant to your mammalian cell line of interest. Select for stably transduced cells, if desired.
5. Assay for recombinant protein of interest.

Methods

General Information

Introduction

The ViraPower™ HiPerform™ Lentiviral Expression System is designed to help you create a lentivirus to deliver and express a gene of interest in mammalian cells. Although the system has 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 who are familiar with the principles of retrovirus biology and retroviral vectors. We highly recommend that users possess a working knowledge of virus production and tissue culture techniques.

For more information about these topics, refer to the following published reviews:

- Retrovirus biology and the retroviral replication cycle: see Buchschacher and Wong-Staal (2000) and Luciw (1996) (Buchschacher & Wong-Staal, 2000; Luciw, 1996)
- Retroviral and lentiviral vectors: see Naldini (1999), Naldini (1998), Yee (1999) and (Pandya *et al*., 2001)

Positive Control

We recommend including a positive control vector in your cotransfection experiment to generate a control lentiviral stock to help optimize expression conditions in your mammalian cell line of interest.

- Each pLenti expression vector kit includes a positive control vector for use as an expression control (e.g., pLenti6.3/V5-TOPO/*lacZ* or pLenti6.3/V5-GW/*lacZ*). For more information about the positive control vector supplied with each kit, refer to the appropriate expression vector manual.
- A control lentiviral expression vector containing Emerald Green Fluorescent Protein (EmGFP) for fluorescent detection (pLenti6.3/V5-GW/EmGFP) is available separately (page 44).

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Lipofectamine® 2000

The Lipofectamine® 2000 reagent supplied with the kit is a proprietary, cationic lipid-based formulation suitable for the transfection of nucleic acids into eukaryotic cells (Ciccarone *et al.*, 1999). Using Lipofectamine® 2000 to transfect 293FT cells offers the following advantages:

- Provides the highest transfection efficiency in 293FT cells.
- DNA-Lipofectamine® 2000 complexes can be added directly to cells in culture medium in the presence of serum.
- Removal of complexes or medium change or addition following transfection are not required, although complexes can be removed after 4 to 6 hours without loss of activity.

Note: Lipofectamine® 2000 is available separately or as part of the ViraPower™ HiPerform™ Lentiviral Support Kits (see page 44).

Opti-MEM® I

To facilitate optimal formation of DNA-Lipofectamine® 2000 complexes, we recommend using Opti-MEM® I Reduced Serum Medium (see page 44).

Generating Your pLenti Expression Construct

Introduction

To generate a pLenti expression construct containing your gene of interest, refer to your specific vector's manual for instructions. After you have created your expression construct, isolate plasmid DNA for transfection.

Important: You should verify that your lentiviral plasmid has not undergone aberrant recombination by performing an appropriate restriction enzyme digest. See the vector manual for details.

DNA Isolation Guidelines

Plasmid DNA for transfection into eukaryotic cells must be clean and free from contamination with phenol and sodium chloride contamination. Contaminants may kill the cells, and salt interferes with lipid complexing, decreasing transfection efficiency.

When using commercially available kits to isolate plasmid DNA from *E. coli* strains (such as Stbl3™) that are wild type for endonuclease 1 (*end*A1+), ensure that Solution I of the Lysis or Resuspension Buffer contains 10 mM EDTA. EDTA inactivates the endonucleases and prevents DNA nicking and vector degradation. Alternatively, follow the instructions included the plasmid purification kits for *end*A1+ *E. coli* strains.

Important

Do not use mini-prep plasmid DNA for lentivirus production. We recommend preparing lentiviral plasmid DNA using the PureLink™ HiPure Plasmid MidiPrep Kit, which contains 10 mM EDTA in the Resuspension Buffer (page 44).

Producing Lentivirus in 293FT Cells

Introduction

Before creating a stably transduced cell line expressing your gene of interest, you first need to produce a lentiviral stock (containing the packaged pLenti expression construct) by cotransfecting the optimized packaging plasmid mix and your pLenti expression construct into the 293FT Cell Line. This section provides protocols and instructions for generating a lentiviral stock.

ViraPower™ Packaging Mix

The pLP1, pLP2, pLP/VSVG plasmids are provided as an optimized mixture to facilitate viral packaging of your pLenti expression vector following cotransfection into 293FT producer cells. The amount of the packaging mix (195 µg) and Lipofectamine® 2000 transfection reagent (0.75 mL) supplied in the ViraPower™ Lentiviral Expression kit is sufficient to perform 20 cotransfections in 10-cm plates.

Note: ViraPower™ Packaging Mix is available separately or as part of the ViraPower™ Lentiviral Support Kits (page 44).

293FT Cell Line

The human 293FT Cell Line, supplied with the ViraPower™ HiPerform™ Lentiviral Expression kits, facilitates optimal lentivirus production (Naldini *et al.*, 1996). The 293FT Cell Line, a derivative of the 293F Cell Line, stably and constitutively expresses the SV40 large T-antigen from pCMVSPORT6TAg.neo and must be maintained in medium containing Geneticin® (page 44).

For more information about pCMVSPORT6TAg.neo and how to culture and maintain 293FT cells, refer to the 293FT Cell Line manual. This manual is supplied with the ViraPower™ HiPerform™ Lentiviral Expression kits, and is also available at www.invitrogen.com or by contacting **Technical Support** (page 45).

Note: The 293FT Cell Line is also available separately (see page 44).

The health of your 293FT cells at the time of transfection is critical to the success of lentivirus production. Use of "unhealthy" cells can 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 293FT cells before use in transfection:

- Make sure that cells are greater than 90% viable.
- Subculture and maintain cells in complete medium containing 0.1 mM MEM Non-Essential Amino Acids, 2 mM L-Glutamine, 1 mM sodium pyruvate, 500 µg/mL Geneticin® and 10% fetal bovine serum that is not heat-inactivated (page 44).

  Note: D-MEM already contains 4 mM L-Glutamine, which is enough to support growth of 293FT cells. However, since L-Glutamine slowly decays over time, supplement the medium with 2 mM L-Glutamine. 293FT cells grow well in 6 mM L-Glutamine, but higher concentrations of L-Glutamine may reduce growth.
- Use cells that have been subcultured for less than 16 passages..

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Producing Lentivirus in 293FT Cells, Continued

Recommended Transfection Conditions

We produce lentiviral stocks in 293FT cells using the **optimized** transfection conditions shown in the table below. The amount of lentivirus produced using these recommended conditions (10 mL of virus at a titer of at least 1×10^5 transducing units (TU)/mL) is generally sufficient to transduce at least 1×10^6 cells at a multiplicity of infection (MOI) value of 1. For example, 10 wells of cells plated at 1×10^5 cells/well in 6-well plates could each be transduced with 1 mL of a 1×10^5 TU/mL virus stock to achieve an MOI of 1.

Condition	Amount
Tissue culture plate size	10 cm (one per lentiviral construct)
Number of 293FT cells to transfect	6×10^6 cells (see **Recommendation** on previous page to prepare cells for transfection)
Amount of ViraPower™ Packaging Mix	9 µg (9 µL of 1 µg/µL stock)
Amount of pLenti expression plasmid	3 µg
Amount of Lipofectamine® 2000 Reagent	36 µL

Note: You may produce lentiviral stocks using other tissue culture formats; optimization may be necessary to obtain the expected titers.

Recommended Procedure

If you are producing lentivirus for the first time using the ViraPower™ System and 293FT cells, perform the **Forward Transfection** procedure on page 15. This procedure requires plating the 293FT cells the day before transfection to obtain cells that are 90–95% confluent.

Note: In previous ViraPower™ manuals, this protocol was referred to as the Alternate Transfection Method.

If you are an experienced lentivirus user and are familiar with the growth characteristics of 293FT cells, you may perform the **Reverse Transfection** procedure on page 16. In this procedure, 293FT cells are added to media containing the DNA-Lipofectamine® 2000 complexes.

Continued on next page

Producing Lentivirus in 293FT Cells, Continued

Materials Needed

- pLenti expression vector containing your gene of interest (0.1–3.0 µg/µL in sterile water or TE, pH 8.0)
- 293FT cells cultured in the appropriate medium (i.e. D-MEM containing 10% FBS, 2 mM L-Glutamine, 0.1 mM MEM Non-Essential Amino Acids, and 1% penicillin-streptomycin, and 500 µg/mL Geneticin®)

  Note: D-MEM already contains 4 mM L-Glutamine, which is enough to support growth of 293FT cells. However, since L-Glutamine slowly decays over time, supplement the medium with 2 mM L-Glutamine. 293FT cells grow well in 6 mM L-Glutamine, but higher concentrations of L-Glutamine may reduce growth.
- Opti-MEM® I Reduced Serum Medium (pre-warmed to 37°C, page 44)
- Fetal bovine serum (FBS, page 44)
- Complete growth medium **without antibiotics** (i.e. D-MEM containing 10% FBS, 2 mM L-Glutamine, 0.1 mM MEM Non-Essential Amino Acids, and 1 mM MEM sodium pyruvate), pre-warmed to 37°C

  Note: MEM Sodium Pyruvate provides an extra energy source for the cells and is available separately; see page 44. See note above for L-Glutamine concentration.
- Sterile, 10-cm tissue culture plates (one each for the lentiviral construct, positive control, and negative control)
- Sterile, tissue culture supplies
- 15-mL sterile, capped, conical tubes
- Cryovials
- CO_2 humidified incubator set at 37°C
- Centrifuge capable of 2,000 × *g*
- *Optional:* Millex®-HV 0.45-µm PVDF filters or equivalent
- *Optional:* pLenti control vector containing EmGFP (sold separately, page 44)

Components supplied with the kits

- ViraPower™ Packaging Mix
- pLenti control vector containing *lacZ*
- Lipofectamine® 2000 transfection reagent (mix gently before use)

Continued on next page

Forward Transfection Procedure (First-Time Users)

If you are a **first-time user**, follow the procedure below to cotransfect 293FT cells. For information on positive controls, see page 9. Include a negative control (no DNA, no Lipofectamine® 2000) in your experiment to help evaluate results.

1. The day before transfection (Day 1), plate 293FT cells in a 10-cm tissue culture plate so that they will be 90–95% confluent on the day of transfection (i.e. 5×10^6 cells in 10 mL of growth medium containing serum). **Do not include antibiotics in the medium.** Incubate the cells overnight at 37°C in a humidified 5% CO_2 incubator.
2. On the day of transfection (Day 2), remove and discard the culture medium from the 293FT cells and replace it with 5 mL of growth medium (Opti-MEM® I Medium, page 44) containing serum. **Do not use antibiotics in the medium.**
3. **For each transfection sample**, prepare DNA-Lipofectamine® 2000 complexes:
   a. In a sterile 5-mL tube, dilute 9 µg of the ViraPower™ Packaging Mix and 3 µg of your pLenti expression plasmid DNA (12 µg total) in 1.5 mL of Opti-MEM® I Medium without serum. Mix gently.
   b. In a separate, sterile 5-mL tube, dilute 36 µL of Lipofectamine® 2000 (mix gently before use) in 1.5 mL of Opti-MEM® I Medium without serum. Mix gently and incubate for 5 minutes at room temperature.
   c. After incubation, combine the diluted DNA (from Step a) with the diluted Lipofectamine® 2000 (from Step b). Mix gently.
   d. Incubate the tube 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 all the DNA-Lipofectamine® 2000 complexes dropwise to the plate of 293FT cells (Steps 1 and 2). Mix gently by rocking the plate back and forth. Incubate the cells overnight at 37°C in a humidified 5% CO_2 incubator.
5. The next day (Day 3), remove the cell culture plate containing the 293FT cells with DNA-Lipofectamine® complexes from the incubator. Remove and discard the medium containing the DNA-Lipofectamine® 2000 complexes and replace it with 10 mL of complete culture medium **without antibiotics**.
6. Incubate the cells for 48–72 hours at 37°C in a humidified 5% CO_2 incubator. (Collecting the supernatant at 48 or 72 hours post-transfection makes minimal difference in viral yield.)

   Note: Expression of the VSV G glycoprotein causes 293FT cells to fuse, resulting in the appearance of large, multinucleated cells known as syncytia. This morphological change is normal and does not affect lentivirus production.
7. Post-transfection (Day 5 or 6), harvest virus-containing supernatants by removing and transferring the medium into a 15 mL sterile, capped, conical tube.

   Caution: Remember that you are working with infectious virus at this stage. Follow recommended guidelines for working with BL-2 organisms (refer to page 7).
8. Centrifuge the supernatants at 2,000 × *g* for 15 minutes at 4°C to pellet debris.
9. *Optional:* Filter the viral supernatants through a Millex®-HV 0.45-µm, or an equivalent, PVDF filter (see **Note**, page 17).
10. Pipet viral supernatants into cryovials in 1-mL aliquots. Store viral stocks at −80°C. Proceed to **Titering Your Lentiviral Stock**, page 18.

Continued on next page

Reverse Transfection Procedure (Experienced Users)

If you are an **experienced user**, you may use the reverse transfection procedure to cotransfect 293FT cells. For information on positive controls, see page 9. Include a negative control (no DNA, no Lipofectamine® 2000) in your experiment to help evaluate results. You will need 6×10^6 293FT cells for each sample.

1. On Day 1, prepare DNA-Lipofectamine® 2000 complexes **for each transfection sample** as follows:
   a. In a sterile 5-mL tube, dilute 9 µg of the ViraPower™ Packaging Mix and 3 µg of your pLenti expression plasmid DNA (12 µg total) in 1.5 mL of Opti-MEM® I Medium without serum. Mix gently.
   b. In a separate sterile 5-mL tube, dilute 36 µL of Lipofectamine® 2000 (mix gently before use) in 1.5 mL of Opti-MEM® I Medium without serum. Mix gently and incubate for 5 minutes at room temperature.
   c. After incubation, combine the diluted DNA (from Step a) with the diluted Lipofectamine® 2000 (from Step b). Mix gently.
   d. Incubate the tube 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.
2. While DNA-lipid complexes are forming, trypsinize and count the 293FT cells. Resuspend the cells at a density of 1.2×10^6 cells/mL in growth medium (or Opti-MEM® I Medium) containing serum. **Do not include antibiotics in the medium.**
3. Add the DNA-Lipofectamine® 2000 complexes (from Step 1d) to a 10-cm tissue culture plate containing 5 mL of growth medium (or Opti-MEM® I Medium) containing serum. **Do not include antibiotics in the medium.**
4. Add 5 mL of the 293FT cell suspension from Step 2 (6×10^6 total cells) to the plate containing media and DNA-Lipofectamine® 2000 complexes (from Step 3). Mix gently by rocking the plate back and forth. Incubate cells overnight at 37°C in a humidified 5% CO_2 incubator.
5. The next day (Day 2), remove and discard the medium containing the DNA-Lipofectamine® 2000 complexes and replace it with 10 mL of complete culture medium **without antibiotics**.
6. Incubate the cells for 48–72 hours at 37°C in a humidified 5% CO_2 incubator. (The difference in viral yield is minimal whether the supernatants are collected 48 or 72 hours after transfection.)

   Note: Expression of the VSV G glycoprotein causes 293FT 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.
7. Posttransfection (Day 4 or 5), harvest virus-containing supernatants by removing and placing the medium into a 15-mL sterile, capped, conical tube.

   Caution: Remember that you are working with infectious virus at this stage. Follow recommended guidelines for working with BL-2 organisms (refer to page 7).
8. Centrifuge supernatants at 2,000 × *g* for 15 minutes at 4°C to pellet debris.
9. *Optional:* Filter the viral supernatants through a Millex®-HV 0.45-µm, or equivalent, PVDF filter (see **Note**, next page).
10. Pipet viral supernatants into cryovials in 1-mL aliquots and store them at −80°C. Proceed to **Titering Your Lentiviral Stock**, page 18.

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Note

It should be possible to use the new ViraPower™ HiPerform™ Lentiviral vector constructs for *in vivo* applications; however, we have not yet tested the new constructs *in vivo*.

If you plan to use your lentiviral construct for *in vivo* applications, filter your viral supernatant through a sterile, 0.45-µm low protein binding filter after the low-speed centrifugation step (Step 8, **Reverse Transfection Procedure (Experienced Users)**) to remove any remaining cellular debris. We recommend using Millex®-HV 0.45 µm PVDF filters (Millipore, Cat. no. SLHV033RB) for filtration.

If you wish to concentrate your viral stock to obtain a higher titer, perform the filtration step prior to concentrating your viral stock.

Concentrating Virus

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 a relatively high Multiplicity of Infection (MOI), 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).

Long-Term Storage

Place lentiviral stocks at −80°C for long-term storage. Repeated freezing and thawing may result in loss of viral titer and is not recommended. When stored properly, viral stocks of an appropriate titer are suitable for use for up to one year. After long-term storage, re-titer viral stocks before transducing your mammalian cell line of interest.

Scaling Up Virus Production

You may scale up the cotransfection experiment to produce a larger volume of lentivirus. For example, we have scaled up the cotransfection experiment from a 10-cm plate to a T-175 cm^2 flask and harvested up to 30 mL of viral supernatant. To scale up your cotransfection, increase the number of cells plated and the amounts of DNA, Lipofectamine® 2000, and medium used in proportion to the difference in surface area of the culture vessel.

Titering Your Lentiviral Stock

Introduction

Before proceeding to transduction and expression experiments, we highly recommend determining the titer of your lentiviral stock. While this procedure is not required for some applications, it is necessary if:

- You wish to control the number of integrated copies of the lentivirus
- You wish to generate reproducible expression results

This section provides guidelines and protocols for titering your lentiviral stock.

In addition to higher expression of the gene of interest, all ViraPower™ HiPerform™ Lentiviral vectors yield a higher Blasticidin (Bsd) or Emerald Green Fluorescence (EmGFP) titer compared to previous pLenti vectors.
The pLenti6.3 vectors (K5310-00 and K5330-00) contain Bsd in the vector backbone, which allows titer of active virus by selection of Blasticidin resistant clones after transduction. Alternatively, pLenti7.3 FastTiter™ vectors (K5320-00 and K5340-00) contain the EmGFP reporter gene in the vector backbone which allows titer by flow cytometry in only **2 days** post-transduction.

For **Titering lentiviral stock using EmGFP**, refer to page 21.

For **Titering lentiviral stock using Blasticidin**, refer to page 25.

ViraPower™ HiPerform™ Lentiviral FastTiter™ Expression Kits

ViraPower™ HiPerform™ Lentiviral FastTiter™ Expression kits (K5320-00 and K5340-00) allow you to titer lentivirus in only **2 days** because the pLenti7.3 vectors contain EmGFP reporter gene in the vector backbone, instead of Bsd. This feature makes these kits ideal for high-throughput and quick-screens of transient expression using flow cytometry.

Important: The FastTiter™ Expression kits are optimal for quick-screens of transient expressions using flow cytometry. The signal intensity produced by these kits is **not** optimal for detection using fluorescence microscopy.
We recommend flow cytometry to detect the EmGFP in your transduced cells.

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Titering Your Lentiviral Stock, Continued

Factors Affecting Viral Titer

A number of factors can influence viral titers, including:

- **The size of your gene of interest.** Titers decrease as the size of the insert increases. We have determined that virus titer drops approximately 2-fold for each kb over 4 kb of insert size. To produce a lentivirus with an insert of > 4 kb, concentrate the virus to obtain a suitable titer (see page 17). The size of the wild-type HIV genome is approximately 10 kb. Because the size of the elements required for expression from pLenti vectors total approximately 4–4.4 kb, the size of your insert should not exceed 5.6 kb.
- **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 may also be used. In general, these cells should belong to an adherent, non-migratory cell line, and exhibit a doubling time in the range of 18–25 hours.
- **The age of your lentiviral stock.** 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, titer your lentiviral stock prior to use.
- **Number of freeze/thaw cycles.** 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.

Selecting a Cell Line for Titering

We strongly recommend the human fibrosarcoma line HT1080 (ATCC, Cat no. CCL-121) as the "gold standard" for reproducibly titering lentivirus. However, you may use the same mammalian cell line that you use for your expression studies to titer your lentiviral stocks (e.g., if you are performing expression studies in a dividing cell line or a non-primary cell line). If you have more than one lentiviral construct, titer all of the lentiviral constructs using the same mammalian cell line. For more information on cells used for titering, see **Factors Affecting Viral Titer**, above.

Using Polybrene® During Transduction

Lentivirus transduction may be enhanced if cells are transduced in the presence of hexadimethrine bromide (Polybrene®, Sigma-Aldrich, Cat. no. H9268). 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, test your cell line for sensitivity to Polybrene® at a range of 0 to 10 µg/mL. If your cells are sensitive to Polybrene® (e.g., exhibit toxicity or phenotypic changes), do not add Polybrene® during transduction.

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Preparing and Storing Polybrene®

Follow the instructions below to prepare Polybrene® (Sigma-Aldrich, Cat. no. H9268):

1. Prepare a 6-mg/mL stock solution in deionized, sterile water.
2. Filter-sterilize and dispense 1-mL aliquots into sterile microcentrifuge tubes.
3. Store stock solutions at −20°C for up to 1 year. Do not freeze/thaw the stock solution more than 3 times to avoid loss of activity.

   Note: The working stock may be stored at 4°C for up to 2 weeks.

Titering Your Lentiviral Stock Using EmGFP

Introduction

This section provides guidelines and protocols for titering your lentiviral stock using Emerald Green Fluorescence (EmGFP).

To titer your lentiviral stock using Blasticidin, refer to page 25.

Remember that you will be working with media containing infectious virus. Follow the recommended Federal and institutional guidelines for working with BL-2 organisms.

- Perform all manipulations within a certified biosafety cabinet.
- Treat media containing virus with bleach before disposal.
- Treat used pipettes, pipette tips, and other tissue culture supplies with bleach and dispose of as biohazardous waste.
- Wear gloves, a laboratory coat, and safety glasses or goggles when handling viral stocks and media containing virus.

Experimental Outline

To determine the titer of your EmGFP lentiviral stocks, you will:

1. Prepare a 50-fold or 20-fold serial dilution of your lentiviral stocks.
2. Transduce the different dilutions of lentivirus in the presence of the polycation Polybrene® (page 19).
3. Determine the Lentiviral titer by fluorescence detection using flow cytometry, 2 days after transduction; see **Important**, below.

Important

We do not recommend using fluorescence microscopy to detect EmGFP in your cells from the pLenti7.3 vectors (we recommend flow cytometry). The pLenti7.3 vectors are designed with EmGFP in their vector backbone, which allows for quick-screens of transient expression in your cells and titering times of only 2-days. While the quantity of cells expressing your gene of interest is significantly greater than other pLenti vectors that do not contain the WPRE and cPPT elements, the signal intensity of EmGFP expressed in your cells is not optimal for viewing with fluorescence microscopy. For this reason, we recommend flow cytometry.

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Titering Your Lentiviral Stock Using EmGFP, Continued

Materials Needed

- Your EmGFP lentiviral stock from the pLenti7.3/V5-TOPO® vector or the pLenti7.3/V5-DEST™ Gateway® vector (store at –80°C until use)
- Adherent mammalian cell line of choice
- Complete culture medium for your cell line
- 96-well tissue culture plates
- *Optional:* 6 mg/mL Polybrene® (see page 19)
- *Optional:* TrypLE™ (see page 44 for ordering information), trypsin, or an equivalent cell dissociation solution
- *Optional:* Flow cytometry buffer of choice, such as calcium/magnesium-free Phosphate Buffered Saline containing 1% FBS or BSA.

Trypsin Dissociation Solution

Before proceeding to analysis with flow cytometry, you need to dissociate your cells from the wells. To prepare the dissociation solution using TrypLE™:

1. Dilute TrypLE™ 1:3 in PBS.
2. Add 25 µL of a 1-mg/mL propidium iodide stock solution (see page 44 for ordering information).

Transduction and Titering Procedure for EmGFP

Follow the procedure below to determine the titer of your lentiviral stock. You will use one 96-well plate for each lentiviral stock to be titered.

1. **24 hours before transduction**, seed cells in a 96-well plate at a density of 6,000 cells per well. Incubate the cells in a 37°C CO_2 incubator overnight.
2. On the day of transduction (Day 1), thaw your lentiviral stock. In a biosafety cabinet, prepare a 50-fold or 20-fold serial dilution of the Lentiviral stock in DMEM growth medium supplemented with Polybrene® (page 19). Mix each virus dilution gently by inversion (**DO NOT** vortex).

   Important: Do NOT dilute virus in culture medium containing Blasticidin.
3. Remove the culture medium from each well of cells and replace it with the diluted virus solution. Allocate 3–6 replicate wells per sample.
4. Swirl the plate gently to mix. Incubate the plate at 37°C in a CO_2 incubator overnight.
5. After 24 hours incubation (Day 2), remove the virus-containing medium from each well and discard (See **Caution**, previous page). Replace the spent medium with 100 µL of fresh growth medium in each well and incubate the plate overnight in a 37°C CO_2 incubator.

   Important: Do NOT add Blasticidin to the growth medium.
6. After 24 hours incubation (Day 3), remove the growth medium from each well and discard. Replace the medium in each well with dissociation solution (see above).
7. Allow the cells to dissociate for 5 minutes at 37°C, and then proceed to **Preparing Cells for Flow Cytometry**, next page (see **Important**, previous page).

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Titering Your Lentiviral Stock Using EmGFP, Continued

Note

If you wish to fix your cells before flow cytometry, use 2% formaldehyde or paraformaldehyde solution in calcium/magnesium-free PBS. Note that these fixatives may increase the autofluorescence of the cells; therefore, it is critical to include fixed, mock-transduced cells as a negative control for flow cytometry detection parameters.

Preparing Cells for Flow Cytometry

Prepare your cells for flow cytometry using an FITC filter according to the protocols established at your flow cytometry facility. The steps below provide simple guidelines, and other methods may be suitable.

1. After cell dissociation (Steps 6–7, **Transduction and Titering Procedure for EmGFP**) centrifuge cells at 2,000 × *g* to remove residual media components, and then resuspend the cell pellet in a flow cytometry buffer such as calcium/magnesium-free PBS with 1% FBS at the required density for analysis on your flow cytometer. Fixing the cells is not necessary but may be done (see **Note** above).
2. Use the mock-transduced cells and the lowest dilution of the virus (i.e. 10^{-1}) as negative and positive samples to set up the parameters of your flow cytometer.

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Titering Your Lentiviral Stock Using EmGFP, Continued

Calculating Lentiviral Titer for EmGFP

EmGFP lentivirus titers should be calculated from the dilutions at which the percentage of GFP-positive cells fall within the range of 1–30% (White *et al.*, 1999) (Sastry *et al.*, 2002). This is to avoid analyzing dilution samples containing multiple integrated lentiviral genomes, which may result in an underestimate of the viral titer, or dilution samples containing too few transduced cells, which will give inaccurate results. Titer is expressed as transducing units (TU)/mL.

The following formula (White *et al.*, 1999) (Sastry *et al.*, 2002) is used to calculate the titer:

$$[F \times C/V] \times D$$

F = frequency of GFP-positive cells (percentage obtained divided by 100)

C = total number of cells in the well at the time of transduction

V = volume of inoculum in mL

D = lentivirus dilution

In the following example, an EmGFP lentiviral stock was generated using the protocol on the previous page. The stock was concentrated and the following data were generated after performing flow cytometry:

Lentivirus Dilution	% EmGFP Positive Cells
10^{-2}	91.5%
10^{-3}	34.6%
10^{-4}	4.4%

In the above example, the 10^{-4} dilution is used to calculate the titer because the percentage of EmGFP-positive cells falls into the desired range of 1–30%. The frequency of EmGFP-positive cells is 4.4/100 = 0.044, multiplied by 2×10^5 (the number of cells in the well) divided by 1 (the volume of inoculum). Thus, titer in this example is:

$$[(0.044 \times 200{,}000)/1] \times 10^4 = 8.8 \times 10^7 \text{ TU/mL}.$$

What You Can Expect

We typically obtain unconcentrated EmGFP lentivirus titers in the range of $5 \times 10^5 – 2 \times 10^6$ TU/mL. To obtain higher lentivirus titers, concentrate your virus (see page 17). The titer of concentrated lentivirus stocks may be up to 1×10^8 TU/mL.

Titering Your Lentiviral Stock Using Blasticidin

Introduction

This section provides guidelines and protocols for titering your lentiviral stock using Blasticidin (see page 44 for ordering information).

To titer your lentiviral stock using EmGFP, refer to page 21.

Remember that you will be working with media containing infectious virus. Follow the recommended Federal and institutional guidelines for working with BL-2 organisms.

- Perform all manipulations within a certified biosafety cabinet.
- Treat media containing virus with bleach before disposal.
- Treat used pipettes, pipette tips, and other tissue culture supplies with bleach and dispose of as biohazardous waste.
- Wear gloves, a laboratory coat, and safety glasses or goggles when handling viral stocks and media containing virus.

Experimental Outline

To determine the titer of your lentiviral stocks using Blasticidin, you will:

1. Prepare 10-fold serial dilutions of your lentiviral stocks.
2. Transduce the different dilutions of lentivirus in the presence of the polycation Polybrene® into a mammalian cell line (HT1080 is recommended).
3. Select for stably transduced cells using Blasticidin.
4. Stain and count the number of Blasticidin-resistant colonies in each dilution.

Antibiotic Selection

The pLenti6.3 expression constructs contain the Blasticidin resistance gene (*bsd*) (Kimura *et al.*, 1994) to allow for Blasticidin selection (Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965) of mammalian cells that have stably transduced the lentiviral construct.

Blasticidin is supplied with the ViraPower™ HiPerform™ Lentiviral Expression Kit, and is also available separately. See page 44 for ordering information.

Preparing Blasticidin

For more information about how to prepare and handle Blasticidin, refer to the **Appendix** (page 37).

Continued on next page

Titering Your Lentiviral Stock Using Blasticidin, Continued

Determining Antibiotic Sensitivity

To select for stably transduced cells using Blasticidin, you must first determine the minimum concentration of Blasticidin required to kill your untransduced mammalian cells (i.e. perform a kill curve experiment). Typically, concentrations ranging from 2–10 µg/mL Blasticidin are sufficient to kill most untransduced mammalian cells. Test a range of concentrations (see protocol below) to ensure that you determine the minimum concentration necessary for your cell line.

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

Materials Needed

- Your pLenti lentiviral stock from the pLenti6.3/V5-TOPO® vector or pLenti6.3/V5-DEST™ Gateway® vector (store at –80°C until use)
- Adherent mammalian cell line of choice
- Complete culture medium for your cell line
- *Optional:* 6 mg/mL Polybrene® (see page 19)
- 6-well tissue culture plates
- Crystal violet (Sigma, Cat. no. C3886; prepare a 1% crystal violet solution in 10% ethanol)
- Phosphate-Buffered Saline (PBS; page 44)
- Blasticidin (10 mg/mL stock), as appropriate for selection, (This component is supplied with the ViraPower™ HiPerform™ Lentiviral TOPO® Expression Kit and the ViraPower™ HiPerform™ Lentiviral Gateway® Expression Kit.)

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Titering Your Lentiviral Stock Using Blasticidin, Continued

Transduction and Titering Procedure Blasticidin

Follow the procedure below to determine the titer of your lentiviral stock. 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, trypsinize and count the cells, and then plate them in a 6-well plate at a density of 2×10^5 cells per well, so that they will be 30–50% confluent at the time of transduction. Incubate the cells at 37°C overnight in a humidified 5% CO_2 incubator.

   Example: When using HT1080 cells, plate 2×10^5 cells per well in a 6-well plate.

2. On the day of transduction (Day 1), 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 the dilutions.

   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, see page 19) to each well to a final concentration of 6 µg/mL. Swirl the plate gently to mix. Incubate the plate at 37°C overnight in a humidified 5% CO_2 incubator.
5. The following day (Day 2), remove the virus-containing medium and replace it with 2 mL of complete culture medium. Incubate the plate at 37°C overnight in a humidified 5% CO_2 incubator.
6. The following day (Day 3), remove the medium and replace it with complete culture medium containing the appropriate amount of Blasticidin to select for stably transduced cells.
7. Replace the spent medium with fresh medium containing Blasticidin every 4–5 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 into each well (1 mL for 6-well plate; 5 mL for 10-cm plate) and incubate the plate for 10 minutes at room temperature.
10. Remove the crystal violet solution and wash the cells with PBS. Repeat the wash.
11. Count the blue-stained colonies and determine the titer of your lentiviral stock.

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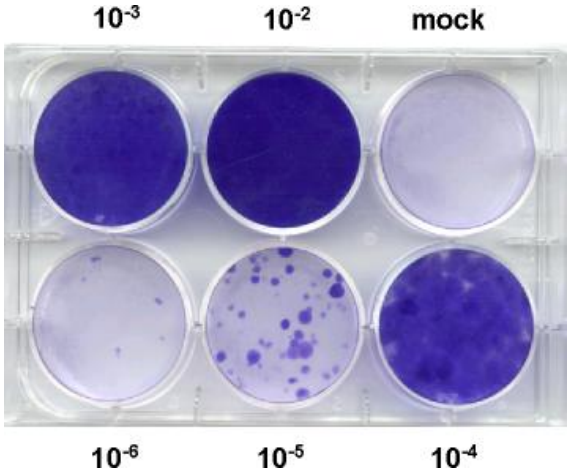
Titering Your Lentiviral Stock Using Blasticidin, Continued

What You Should See

When titering pLenti lentiviral stocks using HT1080 cells, you should expect to obtain titers ranging from 1×10^5 to 5×10^5 (for unconcentrated virus) and up to 2×10^7 (for concentrated virus) transducing units (TU)/mL.

Example of Expected Results

In this experiment, a Lenti6.3/V5-GW/*lacZ* lentiviral stock was generated using the protocol described on page 15 and then concentrated by ultracentrifugation. HT1080 cells were transduced with 10-fold serial dilutions of the lentiviral supernatant (10^{-2} to 10^{-6} dilutions) or untransduced (mock) following the protocol on page 27. At 48 hours after transduction, the cells were placed under Blasticidin selection (10 µg/mL). After 10 days of selection, the cells were stained with crystal violet (see plate below), and colonies were counted.



In the plate above, the colony counts were:

- Mock: no colonies
- 10^{-2} dilution: confluent; undeterminable
- 10^{-3} dilution: confluent; undeterminable
- 10^{-4} dilution: confluent; undeterminable
- 10^{-5} dilution: 46
- 10^{-6} dilution: 5

Thus, the titer of this concentrated lentiviral stock is 4.8×10^6 TU/mL (i.e. the average of 46×10^5 and 5×10^6).

Next Steps

Note that user experience, the nature of the gene, and vector backbone may affect virus titer.

- If the titer of your unconcentrated virus is suitable (i.e. 1×10^5 TU/mL or higher), proceed to **Transduction and Analysis** (page 29).
- If the titer of your concentrated lentiviral stock is less than 1×10^5 TU/mL, produce a new lentiviral stock.

See **Troubleshooting** (page 33) for more tips and guidelines to optimize your viral yield.

Transduction and Analysis

Introduction

After you have generated a lentiviral stock with a suitable titer, transduce the lentiviral construct into a mammalian cell line of choice and assay for expression of your recombinant protein. Guidelines are provided below.

Important

Your lentiviral construct contains a deletion in the 3′ LTR that leads to self-inactivation of the lentivirus after it is transduced into mammalian cells. Once integrated into the genome, the lentivirus can no longer produce packageable virus.

Transient vs. Stable Expression

After transducing your lentiviral construct into a mammalian cell line, you may assay for the expression of your gene of interest in the following ways:

- For pLenti6.3 and pLenti 7.3 vectors, 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 the transduced cells to accumulate the expressed protein.
- For pLenti6.3 vectors **only**, select for stably transduced cells using Blasticidin. This requires a minimum of 10–12 days after transduction, but allows the generation of clonal cell lines that stably express the gene of interest. Be aware that the pLenti7.3 vectors are used for transient expression only and do not produce stably transduced cells.

  Note: We have observed stable expression of a target gene for at least 6 weeks following transduction and selection.

Multiplicity of Infection (MOI)

To obtain optimal expression of your gene of interest, transduce the lentiviral construct into a 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.

Continued on next page

Transduction and Analysis

Determining the Optimal MOI

A number of factors influence optimal MOI, including:

- The nature of your mammalian cell line (e.g., non-dividing vs. dividing cell type; see **Recommendation**, below)
- The transduction efficiency of your mammalian cell line
- The nature of your target gene of interest
- Your application of interest

If you are transducing the lentiviral construct into your mammalian cell line for the first time, use a range of MOIs (e.g., 0, 0.5, 1, 5, 10) to determine the MOI required to obtain the optimal protein expression for your application.

We have found that, in general, 80–90% of the cells in an actively dividing cell line (e.g., HT1080) express a target gene when transduced at an MOI of ~1. Some non-dividing cell types transduce lentiviral constructs less efficiently. For example, only about 50% of the cells in a culture of primary human fibroblasts express a target gene when transduced at an MOI of ~1. If you are transducing your lentiviral construct into a non-dividing cell type, you may need to increase the MOI (e.g., MOI = 10) to achieve optimal expression levels for your recombinant protein.

Positive Control

Control lentiviral vectors expressing *lacZ* are available for optimization (see your vector manual and page 44 for information). If you have generated a lentiviral stock of a *lacZ* expression control (pLenti6.3/V5-GW/*lacZ* or pLenti6.3/V5-GW/EmGFP), use the stock to help you determine the optimal MOI for your particular cell line and application. After you have transduced the control lentivirus into a mammalian cell line, the gene encoding β-galactosidase is constitutively expressed and can be easily assayed (refer to the expression vector or expression control vector manual for assay methods).

Note

Viral supernatants are generated by harvesting spent media containing virus from the 293FT 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 (e.g., 1 mL of viral supernatant per well in a 6-well plate), the 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.

Continued on next page

Transduction and Analysis, Continued

Materials Needed

- Your titered lentiviral stock, page 18 (store at –80°C until use)
- Mammalian cell line of choice
- Complete culture medium for your cell line
- 6 mg/mL Polybrene®, if desired (page 19)
- Appropriately sized tissue culture plates for your application
- Blasticidin, as appropriate (if selecting for stably transduced cells, **pLenti6.3 vectors only**). (Blasticidin is supplied with the ViraPower™ HiPerform™ Lentiviral TOPO® Expression Kit and the ViraPower™ HiPerform™ Lentiviral Gateway® Expression Kit.)

Transduction Procedure for Blasticidin

Follow the procedure below to transduce the mammalian cell line of choice using the pLenti6.3 vectors.

1. Plate the cells in complete medium 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 the virus-containing medium as low as possible to maximize transduction efficiency. **Do not** vortex.
3. Remove the culture medium from the cells. Mix the virus-containing medium gently by pipetting and add it to the cells.
4. Add Polybrene® (if desired) to a final concentration up to 10 µg/mL. Swirl the plate gently to mix and incubate it at 37°C in a humidified 5% CO_2 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, you may incubate the cells for as little as 6 hours before changing the medium.
5. The following day (Day 2), remove the virus-containing medium and replace it with fresh, complete culture medium. Incubate the cells at 37°C in a humidified 5% CO_2 incubator overnight.
6. The following day (Day 3), perform one of the following and then proceed to Step 7:
   - If you are performing transient expression experiments, harvest the cells and assay for expression of your recombinant protein.

   or

   - To select for stably transduced cells, remove the spent medium and replace it with fresh, complete medium containing the appropriate amount of Blasticidin.
7. Replace the spent medium with fresh medium containing Blasticidin every 3–4 days until antibiotic-resistant colonies can be identified (generally 10 to 12 days after selection).
8. Pick at least 5 antibiotic-resistant colonies (see the **Note** on page 32) and expand each clone to assay for expression of the recombinant protein.

Continued on next page

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 observe varying levels of recombinant protein expression from different antibiotic-resistant clones. Test at least 5 antibiotic-resistant clones and select the clone that provides the optimal expression of your recombinant protein for further studies.

Detecting Recombinant Protein

You may use any method to detect your recombinant protein of interest, including functional analysis, immunofluorescence, or western blot. If you have cloned your gene of interest in-frame with an epitope tag, you may easily detect your recombinant protein in a western blot using an antibody specific to the epitope tag (see your lentiviral vector manual for details).

Transduction Procedure for EmGFP

Follow the procedure below to transduce the mammalian cell line of choice using the pLenti7.3 vectors.

1. Plate the cells in complete medium 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 (see **Determining Optimal MOI**, page 30) into fresh complete medium. Keep the total volume of virus-containing medium as low as possible to maximize transduction efficiency.
3. Remove the culture medium from the cells. Mix the virus-containing medium gently by pipetting (**DO NOT** vortex) and add it to the cells.
4. Add Polybrene® (if desired) to the plate at a final concentration of 6 µg/mL. Swirl the plate gently to mix and incubate it at 37°C in a CO_2 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, you may incubate the cells for as little as 6 hours before changing the medium.
5. The following day (Day 2), remove the virus-containing medium and replace it with fresh, complete culture medium **without** Blasticidin.
6. The following day (Day 3), analyze the cells for expression of EmGFP by flow cytometry (see **Important,** page 21)
7. You may sort the cells expressing EmGFP with flow cytometry and use these cells for assaying protein expression

Note: Because pLenti7.3 vectors do not contain an antibiotic selection marker, they do not generate antibiotic resistant clones. Although your gene of interest is integrated into the lentiviral genome, there is no antibiotic selection pressure maintaining the integrity of the expression construct. As a result, depending on the influence of surrounding genomic sequences, your construct may change over the course of multiple passages, resulting in reduction or loss of protein expression.

Troubleshooting

Generating the Lentiviral Stock

The table below lists some potential problems and possible solutions that may help you troubleshoot your cotransfection and titering experiments.

Observation	Reason	Solution
Low viral titer	Low transfection efficiency:	
	• Used poor quality expression construct plasmid DNA (i.e. plasmid DNA from a mini-prep)	• **Do not** use mini-prep plasmid DNA for transfection. Use the PureLink™ HiPure Plasmid Midiprep Kit or CsCl gradient centrifugation to prepare plasmid DNA.
	• Unhealthy 293FT cells; cells exhibit low viability	• Use healthy 293FT cells under passage 16; do not overgrow them.
	• Cells transfected in medium containing antibiotics (i.e. Geneticin®)	• Although Geneticin® is required for stable maintenance of 293FT cells, **do not** add Geneticin® to medium during transfection as this reduces transfection efficiency and causes cell death.
	• Plasmid DNA:transfection reagent ratio incorrect	• Use a DNA (in µg):Lipofectamine® 2000 (in µL) ratio ranging from 1:2 to 1:3.
	• Insufficient co-transfection	• Use more DNA/ Lipofectamine® 2000 (keeping the ratios the same). For example, use 5 µg of lentiviral vector, 15 µg of packaging mix, and 60 µL of Lipofectamine® 2000 for transfection.
	• 293FT cells plated too sparsely	• Plate cells such that they are 90–95% confluent at the time of transfection **OR** use the Reverse Transfection protocol (i.e. add cells to media containing DNA-lipid complexes; see page 16).
	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 posttransfection. 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. Harvest no later than 72 hours post-transfection.
	Viral supernatant too dilute	Concentrate your virus (Yee, 1999).

Continued on next page

Generating the Lentiviral Stock, continued

Observation	Reason	Solution
Low viral titer	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 with the characteristics discussed on page 19.
	Gene of interest is large	Viral titers generally decrease as the size of the insert increases. Concentrate the virus if titer is low (see page 17). Inserts larger than 5.6 kb are not recommended.
	Polybrene® not included during titering procedure	Transduce the lentiviral construct into cells in the presence of Polybrene®.
	Lipofectamine® 2000 handled incorrectly	• Store at 4°C. **Do not freeze.** • Mix gently by inversion. **Do not vortex.**
	Using fluorescence microscopy to view EmGFP titer	The signal level of EmGFP in the cells is not optimal for visual evaluation using fluorescence microscopy. We recommend using only flow cytometry to evaluate transduction efficiency.
No colonies obtained upon titering	Too much antibiotic used for selection	Determine the antibiotic sensitivity of your cell line by performing a kill curve experiment. Use the minimum amount of antibiotic 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®.
Titer indeterminable; cells confluent	Too little antibiotic used for selection	Increase amount of antibiotic.
	Viral supernatant not diluted sufficiently	Titer lentivirus using a wider range of 10-fold serial dilutions (e.g., 10^{-2} to 10^{-8}).

Continued on next page

Transducing Mammalian Cells

The table below lists some potential problems and possible solutions that may help you troubleshoot your transduction and expression experiment.

Observation	Reason	Solution
No expression of the gene of interest	Promoter silencing	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.
	Viral stocks stored incorrectly	Aliquot and store stocks at –80°C. Do not freeze/thaw more than 3 times.
	Frozen cells used for expression experiments for pLenti7.3 vector	pLenti7.3 vectors are designed for transient expression. We **do not** recommend using frozen cells for these expression experiments.
Poor expression of the gene of interest	Low transduction efficiency: • Polybrene® not included during transduction • Non-dividing cell type used	• Transduce the lentiviral construct into cells in the presence of Polybrene®. • Transduce your lentiviral construct into cells using a higher MOI.
	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 to 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.

Continued on next page

Transducing Mammalian Cells, continued

Observation	Reason	Solution
Cytotoxic effects observed after transduction	Large volume of viral supernatant used for transduction	• Remove the "spent" media containing virus and replace with fresh, complete media. • Concentrate the virus (Yee, 1999).
	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.

Appendix

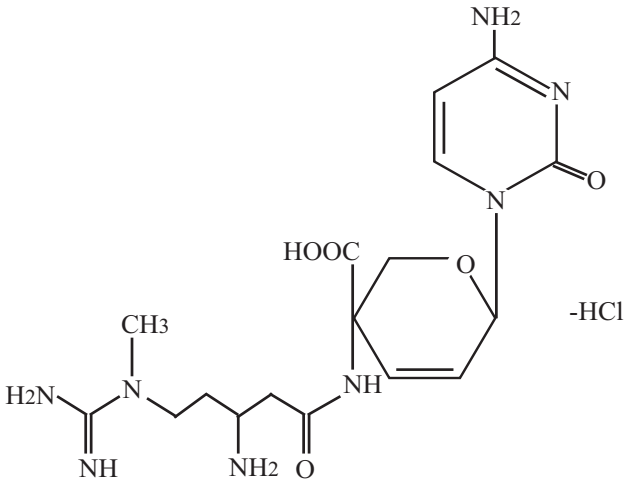
Blasticidin

Description

Blasticidin S HCl is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes* which inhibits protein synthesis in both prokaryotic and eukaryotic cells (Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965). Resistance is conferred by expression of either one of two Blasticidin S deaminase genes: *bsd from Aspergillus terreus* (Kimura *et al.*, 1994) or *bsr* from *Bacillus cereus* (Izumi *et al.*, 1991). These deaminases convert Blasticidin S to a non-toxic deaminohydroxy derivative (Izumi *et al.*, 1991).

Molecular Weight, Formula, and Structure

Merck Index: 12: 1,350
MW: 458.9
Formula: $C_{17}H_{26}N_8O_5$-HCl



Handling Blasticidin

Always wear gloves, mask, goggles, and protective clothing (e.g., a laboratory coat) when handling Blasticidin. Weigh out Blasticidin and prepare solutions in a hood.

Preparing and Storing Stock Solutions

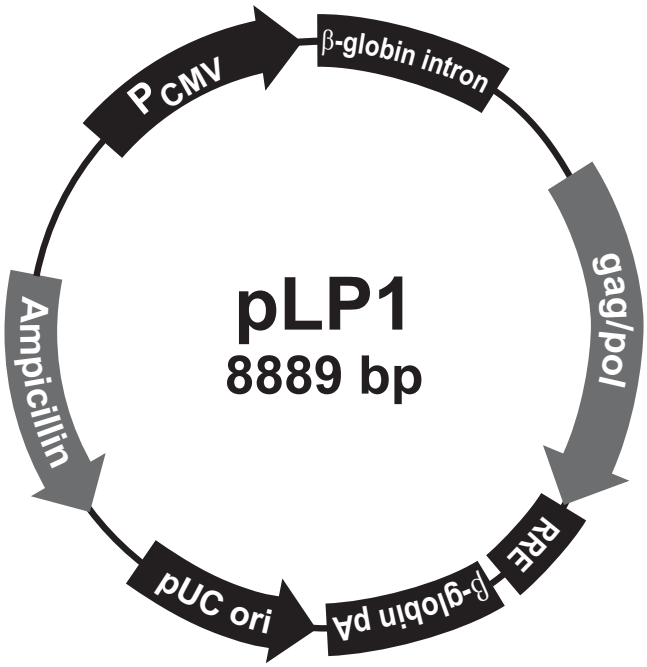
Blasticidin may be obtained in 50-mg aliquots (see page 44)

- Blasticidin is soluble in water and acetic acid.
- Prepare a stock solution of 5 to 10 mg/mL Blasticidin in sterile water and filter-sterilize the solution.
- Aliquot in small volumes suitable for one time use and freeze at –20°C for long-term storage or store at 4°C for short term storage.
- Aqueous stock solutions are stable for 1 week at 4°C and 6–8 weeks at –20°C.
- pH of the aqueous solution should not exceed 7.0 to prevent inactivation of Blasticidin.
- Do not subject stock solutions to freeze/thaw cycles (**do not store in a frost-free freezer**).
- Upon thawing, use what you need and discard the unused portion.
- Medium containing Blasticidin may be stored at 4°C for up to 2 weeks.

Map and Features of pLP1

pLP1 Map

The figure below shows the features of the pLP1 vector. Note that the *gag* and *pol* genes are initially expressed as a gag/pol fusion protein, which is self-cleaved by the viral protease into individual Gag and Pol polyproteins. The sequence of pLP1 is available at **www.invitrogen.com** or by contacting Technical Support (see page 45).



Comments for pLP1
8889 nucleotides

CMV promoter: bases 1-747
    TATA box: bases 648-651
Human β-globin intron: bases 880-1320
HIV-1 gag/pol sequences: bases 1355-5661
    gag coding sequence: bases 1355-2857
    gag/pol frameshift: base 2650
    pol coding sequence: bases 2650-5661
HIV-1 Rev response element (RRE): bases 5686-5919
Human β-globin polyadenylation signal: bases 6072-6837
pUC origin: bases 6995-7668 (C)
Ampicillin (*bla*) resistance gene: bases 7813-8673 (C)
bla promoter: bases 8674-8772 (C)

C=complementary strand

Continued on next page

Map and Features of pLP1, Continued

Features of pLP1

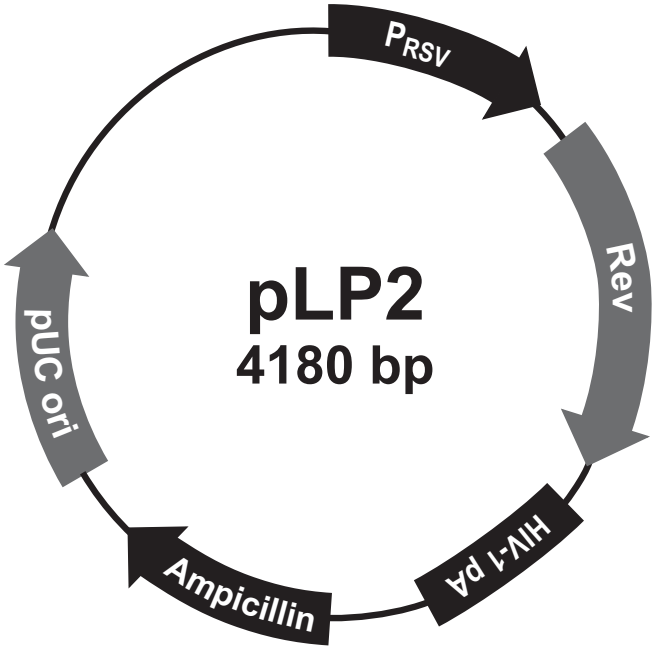
pLP1 (8,889 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) promoter	Permits high-level expression of the HIV-1 *gag* and *pol* genes in mammalian cells (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987).
Human β-globin intron	Enhances expression of the *gag* and *pol* genes in mammalian cells.
HIV-1 *gag* coding sequence	Encodes the viral core proteins required for forming the structure of the lentivirus (Luciw, 1996).
HIV-1 *pol* coding sequence	Encodes the viral replication enzymes required for replication and integration of the lentivirus (Luciw, 1996).
HIV-1 Rev response element (RRE)	Permits Rev-dependent expression of the *gag* and *pol* genes.
Human β-globin polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA.
pUC origin of replication (*ori*)	Permits high-copy replication and maintenance in *E. coli.*
Ampicillin (*bla*) resistance gene	Allows selection of the plasmid in *E. coli.*

Map and Features of pLP2

pLP2 Map

The figure below shows the features of the pLP2 vector. The sequence of pLP2 is available at **www.invitrogen.com** or by contacting Technical Support (see page 45).



Comments for pLP2
4180 nucleotides

RSV enhancer/promoter: bases 1-271
TATA box: bases 200-207
Transcription initiation site: base 229
RSV UTR: bases 230-271
HIV-1 Rev ORF: bases 391-741
HIV-1 LTR polyadenylation signal: bases 850-971
bla promoter: bases 1916-2014
Ampicillin (*bla*) resistance gene: bases 2015-2875
pUC origin: bases 3020-3693

Continued on next page

Map and Features of pLP2, Continued

Features of pLP2

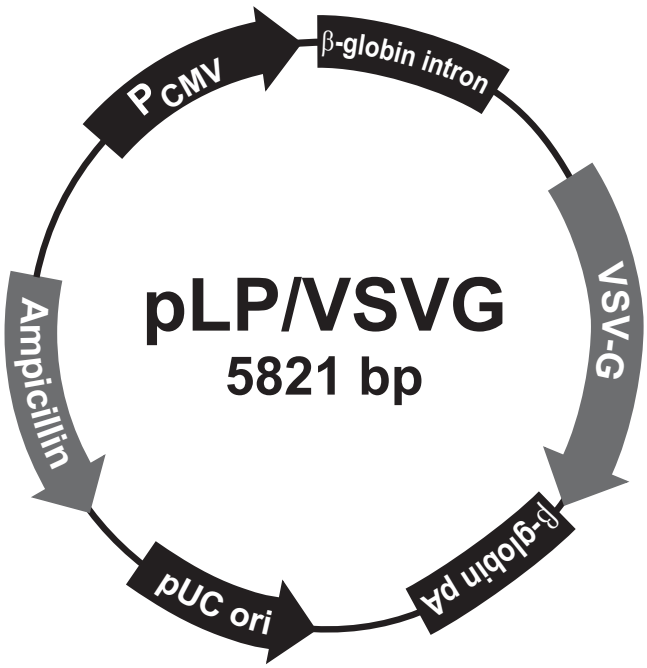
pLP2 (4,180 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
RSV enhancer/promoter	Permits high-level expression of the *rev* gene (Gorman *et al.*, 1982).
HIV-1 Rev ORF	Encodes the Rev protein that interacts with the RRE on pLP1 and on the pLenti6/BLOCK-iT™-DEST expression vector to induce *gag* and *pol* expression, which promotes the nuclear export of the unspliced viral RNA for packaging into viral particles.
HIV-1 LTR polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA.
Ampicillin (*bla*) resistance gene	Allows selection of the plasmid in *E. coli.*
pUC origin of replication (*ori*)	Permits high-copy replication and maintenance in *E. coli.*

Map and Features of pLP/VSVG

pLP/VSVG Map

The figure below shows the features of the pLP/VSVG vector. The sequence of pLP/VSVG is available at www.invitrogen.com or by contacting Technical Support (see page 45).



Comments for pLP/VSVG
5821 nucleotides

CMV promoter: bases 1-747
TATA box: bases 648-651
Human β-globin intron: bases 880-1320
VSV G glycoprotein (VSV-G): bases 1346-2881
Human β-globin polyadenylation signal: bases 3004-3769
pUC origin: bases 3927-4600 (C)
Ampicillin (*bla*) resistance gene: bases 4745-5605 (C)
bla promoter: bases 5606-5704 (C)

C=complementary strand

Continued on next page

Map and Features of pLP/VSVG, Continued

Features of pLP/VSVG

pLP/VSVG (5,821 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Human CMV promoter	Permits high-level expression of the VSV-G gene in mammalian cells (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987).
Human β-globin intron	Enhances expression of the VSV-G gene in mammalian cells.
VSV G glycoprotein (VSV-G)	Encodes the envelope G glycoprotein from Vesicular Stomatitis Virus to allow production of a pseudotyped retrovirus with a broad host range (Burns *et al.*, 1993; Emi *et al.*, 1991; Yee *et al.*, 1994).
Human β-globin polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA.
pUC origin of replication (*ori*)	Permits high-copy replication and maintenance in *E. coli*.
Ampicillin (*bla*) resistance gene	Allows selection of the plasmid in *E. coli*.

Additional Products

Additional Products

Many of the reagents supplied in the ViraPower™ HiPerform™ Lentiviral Expression Kits, as well as other products suitable for use with the kits, are available separately. Ordering information for these reagents is provided below. For more information, go to www.invitrogen.com or contact Technical Support (see page 45).

Product	Amount	Cat. no.
pLenti6.3/V5 TOPO® TA Cloning® Kit	20 reactions	K5315-20
pLenti7.3/V5-TOPO® TA Cloning® Kit	20 reactions	K5325-20
pLenti6.3/V5-DEST™ Gateway® Vector Kit	6 µg	V533-06
pLenti7.3/V5-DEST™ Gateway® Vector Kit	6 µg	V534-06
Vivid Colors™ pLenti6.3/V5-GW/EmGFP Expression Control Vector	20 µg	V370-06
ViraPower™ Lentiviral Packaging Mix	60 reactions	K4975-00
PureLink™ HiPure Plasmid Midiprep Kit	25 reactions	K2100-04
	50 reactions	K2100-05
One Shot® Stbl3™ Chemically Competent *E. coli*	20 × 50 µL	C7373-03
293FT Cell Line	3×10^6 cells, frozen	R700-07
Lipofectamine® 2000	0.75 mL	11668-027
	1.5 mL	11668-019
Opti-MEM® I Reduced Serum Medium	100 mL	31985-062
	500 mL	31985-070
Dulbecco's Modified Eagle Medium (D-MEM)	500 mL	11965-092
	1000 mL	11965-084
TrypLE™ Select (1X), liquid	500 mL	12563-029
TrypLE™ Select Animal-Origin-Free Trypsin-Like Enzyme	100 mL	12563-011
Propidium Iodide (1.0 mg/mL)	10 mL	P-3566
Blasticidin	50 mg	R210-01
Geneticin®	20 mL	10131-035
	100 mL	10131-027
Fetal Bovine Serum (FBS), Certified	500 mL	16000-044
Phosphate-Buffered Saline (PBS), pH 7.4	500 mL	10010-023
	1 L	10010-031

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

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Continued on next page

Purchaser Notification, Continued

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Gateway® Clone Distribution Policy

Introduction

The information supplied in this section is intended to provide clarity concerning Invitrogen's policy for the use and distribution of cloned nucleic acid fragments, including open reading frames, created using Invitrogen's commercially available Gateway® Technology.

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Additional Terms and Conditions

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