Description

Lenti-Fuse™ Polybrene Viral Transduction Enhancer is an easy to use preformulated polybrene solution designed to enhance the transduction efficiency of retroviruses or lentiviruses. Polybrene is a cationic polymer that acts by neutralizing the charge repulsion between the virus and the cell surface (both negatively charged), thus increasing the transduction efficiency.

Background:

Transduction is defined as the process by which foreign DNA is introduced into a cell by a virus or a viral vector. This method of DNA delivery was discovered in 1952 in *Salmonella* and transduction with viral vectors is now an essential tool in molecular biology. Four different types of viral vectors are commonly used: retrovirus, lentivirus, adenovirus and adeno-associated virus. The use of transduction enhancers is common practice as a method to increase efficiency, particularly in cell types known to be hard to transduce, such as primary cells, by improving the cell-virus contact. Both the surface of viral particles and the sialic acid on the cell plasma membrane are negatively charged, which decreases the chance of contact and entrance of the viruses into the cells. Cationic compounds like Polybrene (hexadimethrine bromide) can neutralize the negative charges and allow a closer proximity between the cells and the viruses. The use of transduction enhancers has become an essential tool in cell and gene therapy.

Applications:

Retroviral and lentiviruses-mediated transduction.

Supplied Materials

Components	Format
1 vial	Each vial contains 500 µl at 10 mg/ml in water.

Materials Required but Not Supplied

These materials are not supplied with Lenti-Fuse™ Polybrene Viral Transduction Enhancer but are necessary to follow the protocol described in the "Validation Data" section. Media and reagents used at BPS Bioscience are all validated and optimized for use with this product and are highly recommended for best results.

Name	Ordering Information
HEK293 Cells	ATCC #CRL-1573
Thaw Medium 1	BPS Bioscience #60187
Jurkat Cells	ATCC #TIB-152
Thaw Medium 2	BPS Bioscience #60184
6-well tissue culture plate	
Lentivirus of interest	

Storage

Store at -20°C for up to 2 years.

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Protocol

- The following protocols are general guidelines for transducing HEK293 cells (Adherent Cell Protocol) and Jurkat cells (Suspension Cell Protocol) using lentiviruses. The optimal transduction conditions (e.g. MOI, concentration of Lenti-Fuse™ Polybrene Viral Transduction Enhancer, time of assay development) should be optimized according to the cell type and the assay requirements.
- The optimal concentration of Lenti-Fuse™ Polybrene Viral Transduction Enhancer depends on the cell type and may need to be empirically determined. In most of the cell lines, the optimal concentration of Lenti-Fuse™ Polybrene Viral Transduction Enhancer falls in a range of 3-10 µg/ml. *Note: Lenti-Fuse™ Polybrene Viral Transduction Enhancer can be toxic to some cells. If cells are very sensitive to polybrene, omit Lenti-Fuse™ Polybrene Viral Transduction Enhancer during transduction or use other transduction enhancers as alternatives.*

Adherent Cell Protocol

This Protocol is a general guide on how to transduce adherent cell lines, such as HEK293, CHO or HeLa cells.

Day 1:

1. Seed HEK293 cells at a density of ~150,000 cells per well into a 6-well tissue culture plate in 2 ml of Thaw Medium 1.

2. Add appropriate amount of lentivirus of interest to each well.

3. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well to a final concentration of 5 µg/ml.

4. Gently swirl the plate to mix the cells and virus.

5. Incubate the plate at 37°C with 5% CO_2 overnight.

Day 2:

1. Remove from the wells the medium containing the lentivirus.

2. Add 2 ml of fresh Thaw Medium 1 to each well.

   Note: If neither polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 2. The target cells can be incubated with the virus for 48-72 hours before changing medium.

Day 3-4:

1. 48-72 hours post-transduction, the transduced cells are ready for analysis by flow cytometry, Western Blot, RT-PCR or other methods of interest.

Suspension Cell Protocol:
This protocol, spinoculation, is recommended for transducing suspension cells, such as Jurkat, THP-1, PBMC, T cells, etc.

Day 1:

1. Harvest Jurkat cells by centrifugation.
2. Resuspend cells in fresh Thaw Medium 2 and count.
3. Dilute the cells to 5 x 10^5/ml in Thaw Medium 2.
4. Mix 750 µl of the Jurkat cell suspension and appropriate amount of lentivirus in a 1.5-ml Eppendorf tube.
5. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well to a final concentration of 8 µg/ml.
6. Gently mix and incubate the virus with the Jurkat cells for 20 minutes at room temperature in a tissue culture hood.
7. Centrifuge the virus/cells mixture for 30 minutes at 800 *x g* at 32°C.
8. Remove the supernatant (virus containing medium) and resuspend the cell pellet in 3 ml of fresh Thaw Medium 2.
9. Transfer the cells into one well in a 6-well plate.
10. Incubate the plate at 37°C with 5% CO_2 for 48-72 hours.

    Note: If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to remove the lentivirus after spinoculation. Simply resuspend the cells/virus/polybrene after spinoculation and transfer into additional 2 ml of fresh Thaw Medium 2 in one well of a 6-well plate.

Day 3-4:

1. 48-72 hours post-transduction, the transduced cells are ready for analysis by flow cytometry, Western Blot, RT-PCR or other methods of interest.

Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Negative Control Luciferase Lentivirus	79578	500 µl x 2
Firefly Luciferase Lentivirus	79692	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2