**Description**

Membrane-Bound TNFα Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce nearly all types of mammalian cells, including primary and non-dividing cells. These particles result in expression of uncleavable, membrane-bound TNFα (Tumor necrosis factor alpha) driven by an EF1a promoter, and a puromycin selection marker (Figure 1).

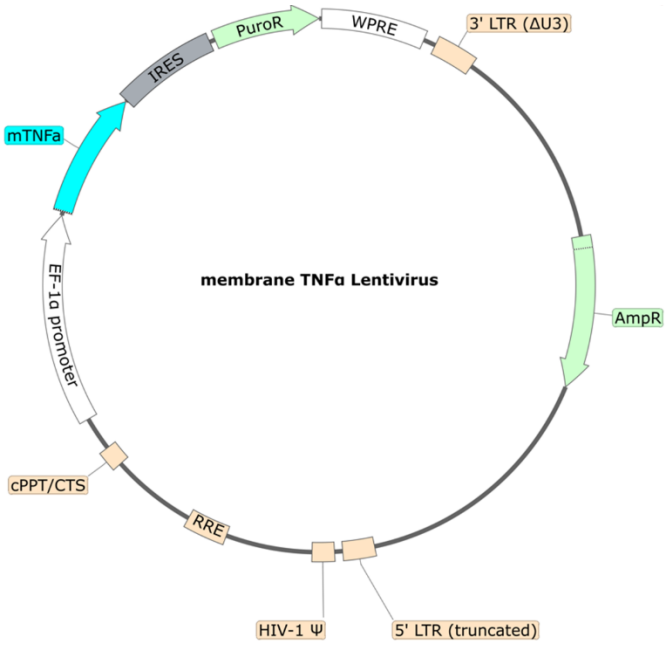


*Figure 1. Schematic of the lenti-vector used to generate Membrane-Bound TNFα (mTNFα) Lentivirus.*

**Background**

Tumor necrosis factor (TNF, also known as TNFα) is a cytokine produced predominantly by activated macrophages and T lymphocytes. It has been identified as a key regulator in inflammatory and immune responses. TNF signaling pathways are triggered by binding to one of two distinct receptors, designated TNFR1 (TNF receptor 1) and TNFR2, which are differentially regulated on various cell types in normal and diseased tissues. TNFα exists in both a trimeric membrane-bound form (mTNFα) and as a soluble protein. TNFα is synthetized in a precursor form, a cell surface type II transmembrane protein, which is cleaved by metalloproteinases such as TACE (TNFα converting enzyme) into a soluble peptide. Soluble TNFα can then bind to its receptors and activate downstream signaling pathways. Transmembrane TNFα can also bind to TNFα receptors and induce cellular responses. For instance, it can enhance cytotoxicity in NK cells, while in the liver it can trigger hepatitis. Anti-TNFα antibodies can bind to mTNFα and trigger antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) to destroy the mTNFα-expressing inflammatory cells, being a promising therapy for inflammatory diseases.

**Application(s)**

- Generate cell pools or stable cell line expressing membrane-bound TNFα following puromycin selection.
- Study anti-TNFα antibodies ADCC (antibody dependent cellular cytotoxicity) potential.

**Formulation**

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations and produced at higher titers by special request, for an additional fee.

**Size and Titer**

Two vials (500 µl x 2) of lentivirus at a titer ≥$10^7$ TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

**Storage**

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

**Biosafety**

The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

**Materials Required but Not Supplied**

These materials are not supplied with this lentivirus 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 lentivirus and are highly recommended for best results.

| Name | Ordering Information |
|---|---|
| Thaw Medium 3 | BPS Bioscience #60186 |
| Lenti-Fuse™ Polybrene Viral Transduction Enhancer | BPS Bioscience #78939 |
| 12-well tissue culture-treated plates | |
| Flow cytometer or fluorescence microscope | |

**Media Formulations**

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

*Media Required for Cellular Assay*

*Thaw Medium 3 (BPS Bioscience #60186):*

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

**Assay Protocol**

- The following protocol is a general guideline for transducing CHO cells. The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the target gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the target with puromycin prior to carrying out the assays.
- The assay should include "Peptide Loaded" and "Unloaded Control" wells.

**Day 1:**

1. Harvest CHO cells from culture and seed cells at a density of 100,000 cells per well into a 12-well microplate in 1 ml of Thaw Medium 3.

2. Incubate the cells at 37°C with 5% $CO_2$ overnight.

**Day 2:**

1. Add 10 µl of Membrane-Bound TNFα (mTNFα) Lentivirus into each well.

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

3. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% $CO_2$ overnight.

   *Note: Alternatively, cell seeding, and transduction can be performed at the same time.*

**Day 3:**

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

   *Note: If neither the polybrene nor the lentiviruses adversely affect the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the viruses for 48-72 hours before changing medium.*

2. Add 1 ml of fresh Thaw Medium 3 to each well.

**Day 4-5:**

1. Approximately 48-72 hours after transduction, the expression of membrane-bound TNFα in the target cells can be assessed by flow cytometry, or another method of interest.

**Notes**

To generate a membrane-bound TNFα expressing stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin (as pre-determined from a killing curve, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.

**Figures and Validation Data**

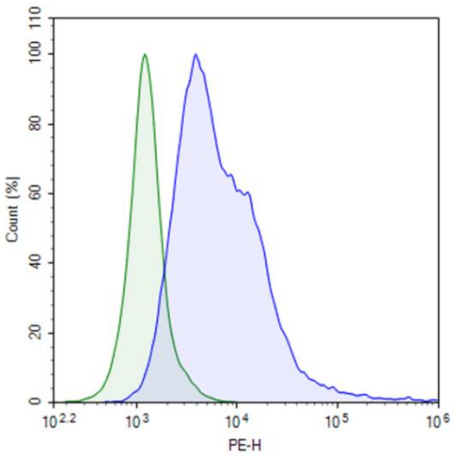


*Figure 2. Expression of membrane-bound TNFα in CHO cells transduced with Membrane-Bound TNFα (mTNFα) Lentivirus by flow cytometry.*
Approximately 100,000 cells/well of CHO cells plated in 12-well plates were transduced with 10 µl/well of Membrane-Bound TNFα (mTNFα) Lentivirus. 48 hours post-transduction the medium was changed for medium containing puromycin. The membrane-bound TNFα CHO cell pool generated (blue) and control parental CHO-K1 cells (green) were stained with Infliximab Recombinant Monoclonal Antibody (ThermoFisher #MA5-41776) followed by Anti-Human IgG Fc, PE (clone: HP6017) (Cedarlane #CL6017PE). The expression of human TNFα was analyzed by flow cytometry. The y axis represents the cell %, while the x axis indicates PE intensity.

*Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

**References**

Wang F., *et al.*, 2017 *Mol Med Rep* 16: 1021-1030.
Horiuchi T., *et al.*, 2010 *Rheumatology* 49(7):1215-1228.

**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* |
|---|---|---|
| Membrane-Bound TNFα CHO Cell Line | 78971 | 2 vials |
| Human Tumor Necrosis Factor-alpha Recombinant | 90244 | 10 µg/50 µg |
| TNFR2 HEK293 Cell Line | 78828 | 2 vials |
| TNFR2 Lentivirus | 78765 | 500 µl x 2 |
| TNFR2, Fc-Fusion (IgG1), His-Avi-Tag Recombinant | 79363 | 100 µg |
| TNFR2:TNFalpha[Biotinylated] Inhibitor Screening Assay Kit | 79756 | 96 reactions |

*Version 032024*