

Description

CD40 Ligand (CD40L) Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce nearly all types of mammalian cells, including primary and non-dividing cells. These viruses result in expression of human CD40L (NM_000074.3) driven by an EF1a promoter, and a puromycin selection marker (Figure 1).

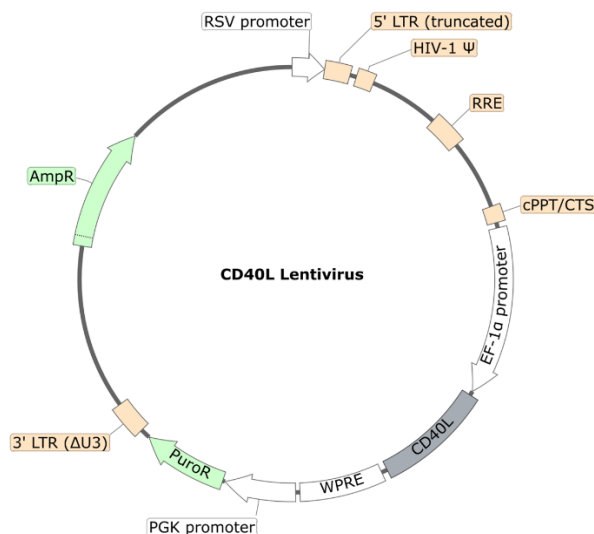


Figure 1. Schematic of the lenti-vector used to generate CD40 Ligand (CD40L) Lentivirus.

Background

CD40L (CD40 ligand), also known as TNFSF5 (tumor necrosis factor receptor superfamily 5) and CD154, is a type II membrane glycoprotein that exists in cells in a membrane bound (mCD40L) and a soluble (sCD40L) form. It is found at high levels in activated CD4⁺ T cells, and at lower levels in Th1, Th2, Th17 and Tregs. Expression can also be induced in NK cells, CD8⁺ T cells, basophils, and others. CD40 and CD40L are stimulatory immune checkpoints, and their signaling is mediated by different TRAF (TNF receptor associated factor), in a cell and stimuli-dependent mode. For example, it mediates the activation of the NF-κB (nuclear factor kappa-B) pathway. The role of CD40 and CD40L as immune checkpoints makes them highly attractive targets in cancer therapy, and several clinical trials using anti-CD40 or anti-CD40L agonist antibodies or trying to increase their expression are underway, targeting both hematological and solid tumors. The inhibition of CD40:CD40L interaction is also clinically relevant, and clinical trials have been focusing on treatment options for lupus, rheumatoid arthritis and ALS (amyotrophic lateral sclerosis). Further studies and development of refined therapies will continue to benefit the cancer therapy field and patients suffering from autoimmune disorders.

Application(s)

- Expression of human CD40L in cells of interest.
- Generate cells pools or stable cell lines expressing CD40L following puromycin selection.

Formulation

The lentivirus particles were produced in HEK293T cells in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

Two vials (500 μl x 2) of lentivirus at a titer $\geq 10^7$ 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.

Notes

To generate an CD40L 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, following by clonal selection.

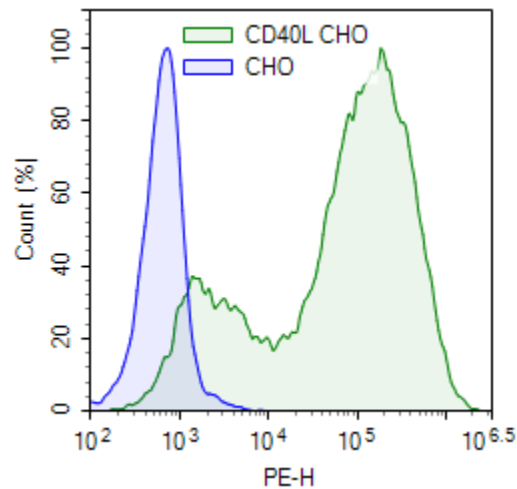
Figures and Validation Data

Figure 2. Expression of CD40L in CHO cells transduced with CD40 Ligand (CD40L) Lentivirus. Approximately 100,000 CHO cells were transduced with 1×10^6 TU ($100 \mu\text{l}$ of 10^7 TU/ml) of CD40 Ligand (CD40L) Lentivirus in the presence of $5 \mu\text{g/ml}$ of Lenti-Fuse™ Polybrene Viral Transduction Enhancer (BPS Bioscience #78939). 66 hours post-transduction, cells were stained with PE anti-CD154 Antibody (Biolegend #310806) and the expression of human CD40L was analyzed by flow cytometry. The y-axis represents the % of 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

Sequence

Human CD40L sequence (NM_000074.3)

MIETYNQTSRSAATGLPISMKIFMYLLTVFLITQMIGSALFAVYLHRRLDKIEDERNLHEDFVFMKTIQRCNTGERSLSLLNCEEIKS
 QFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYTMSNNLVTLENGKQLTVKRQGLYYIYA
 QVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFELQPGASVFNVTDPQSQVSHGTGFTSFGLLK
 L

ReferencesAnnis A., *et al.*, 2004 *J. Amer. Chem. Soc.* 126(4): 15495-15503.Yan, T., *et al.*, 2001 *J. Cellular Biochem.* 83(2): 320-325.Tang T., *et al.*, 2021 *Pharmacol The* 219:107709.**Troubleshooting Guide**

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

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Firefly Luciferase-eGFP Lentivirus (G418 or Puromycin)	79980	500 µl x 2
Expression Negative Control Lentivirus (EF1A Promoter/ Puromycin)	82212-P	500 µl x 2
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	78939	500 µl
CD40:CD40L[Biotinylated] Inhibitor Screening Assay Kit	79257	96 reactions
CD40:CD40L TR-FRET Assay	79258	384 reactions
CD40L (CD154), His-tag (Human) Recombinant	71191	100 µg

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