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Dominant Negative TGF-β Receptor Type II (TGF-βRII) Lentivirus

#78928

Description

Dominant Negative TGF-β Receptor Type II (TGF-βRII) 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 dominant negative TGF-βRII, missing the intracellular kinase domain (NM_003242.6; amino acid 1-191), driven by an EF1a promoter and a puromycin selection marker (Figure 1).

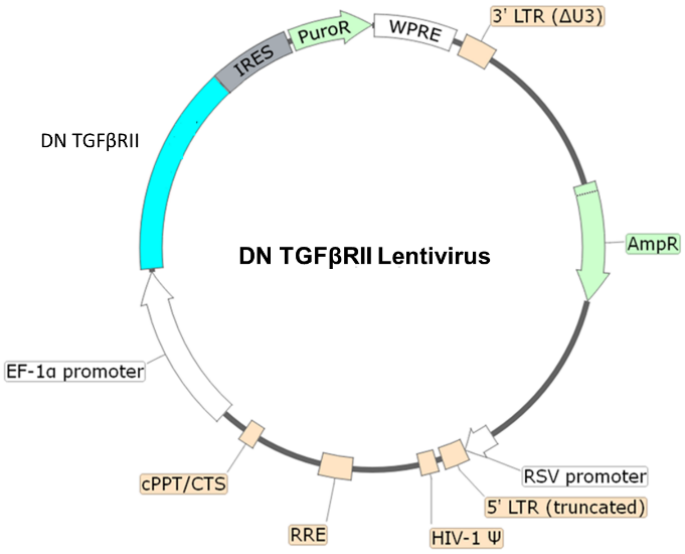

Figure 1. Schematic of the lenti-vector used to generate Dominant Negative TGF-β Receptor Type II (TGF-βRII) Lentivirus.

Background

Transforming growth factor receptor beta 2 (TGFBR2), or TGFβRII, encodes the TGFβ receptor serine/threonine kinase, which is a transmembrane protein that forms a heterodimeric complex with other receptor proteins and binds TGFβ. The association of TGFβ with TGFβRII leads to the phosphorylation of proteins, such as SMADs, involved in cell proliferation, cell cycle arrest, wound healing, and immunosuppression. Dysfunction of the TFGβ signaling tends to result in cancer development and progression. In the case of solid tumors, TGFβ signaling plays a role in creating a highly immunosuppressive TME (tumor microenvironment), restricting the efficacy of CAR (chimeric receptor antigen)- T cells, which have proved successful in the treatment of hematological cancers. Recently, the use of CAR-T cells armored with a dominant negative form of TGFβRII, missing the intracellular kinase domain, for the treatment of prostate cancer resulted in promising outcomes. 5 out of 13 patients did suffer cytokine release syndrome, which continues to be a concern with CAR-T applications, but on the whole the use of a dominant negative TGFβ receptor to armor CAR-T cells appears to be an approach that deserves further attention in the cancer therapy field.

Application(s)

- Expression of human dominant negative TGF-βRII in cells of interest.
- Generate cell pools or stable cell lines expressing human TGF-βRII 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 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.

Notes

To generate a dominant negative TGF-βRII 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

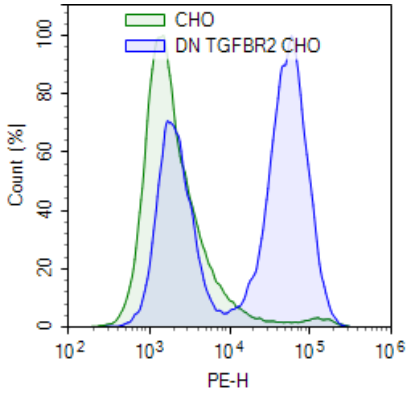

Figure 2. Expression of dominant negative TGF-βRII in CHO cells transduced with Dominant Negative TGF-β Receptor Type II *(TGF-βRII) Lentivirus.*
Approximately 100,000 CHO-K1 cells were transduced with 1 x 10^6 TU (100 µl of 10^7 TU/ml) of Dominant Negative TGF-β Receptor Type II (TGF-βRII) lentiviruses in the presence of 5 µg/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer (BPS Bioscience #78939). 48 hours post-transduction, the cells were cultured with 5 µg/ml of puromycin. The puromycin-resistant cell pool was stained with Human TGF-beta RII PE-conjugated Antibody (R&D Systems FAB2411P) and analyzed by flow cytometry. The y-axis represents the cell % and 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 dominant negative TGF-βRII sequence (accession number NM_003242.6; amino acid 1-191)

MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVC
VAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIFQVTGISL
LPPLGVAISVIIIFYCYRV

References
Ikushima H. and Miyazono K., 2010 *Nature Reviews Cancer* 10:415-424.
Narayan V., *et al.*, 2022 *Nat Med* 28:724-34.

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

Related Products

Products	*Catalog #*	*Size*
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
TGFBR2 CRISPR/Cas9 Lentivirus (Non Integrating)	78536	500 µl x 2
TGFBR2 CRISPR/Cas9 Lentivirus (Integrating)	78535	500 µl x 2
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	78939	500 µl
TGFβ-Responsive SBE Luciferase Reporter HEK293 Cell Line	60653	2 vials

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