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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The PTK7 Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce almost all types of mammalian cells, including primary and non-dividing cells. These viruses transduce cells with human PTK7 (Protein Tyrosine Kinase 7) (NM_002821.5) driven by a CMV promoter. The lentiviruses also transduce a puromycin selection marker (Figure 1).

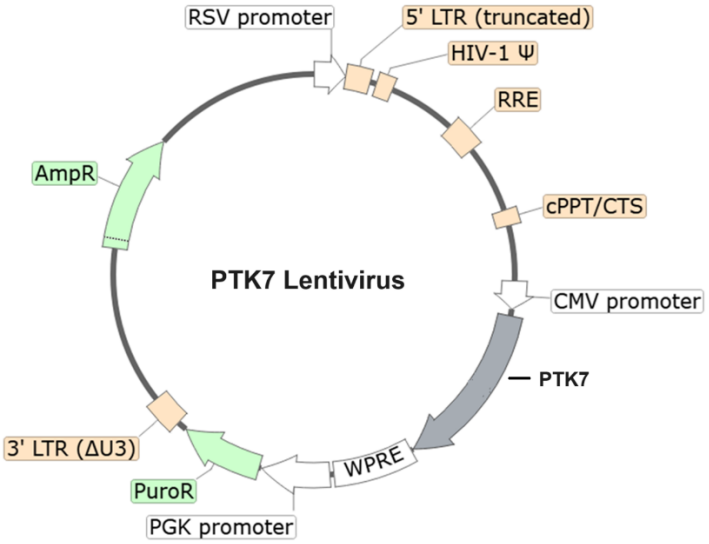

Figure 1. Schematic of the lenti-vector used to generate human PTK7 Lentivirus.

Background

PTK7 (Protein Tyrosine Kinase 7) is a pseudokinase of the receptor tyrosine kinase (RTK) family and shares a common ligand (Wnt5a) with fellow pseudokinases ROR1 (receptor tyrosine kinase-like orphan receptor 1) and ROR2. Despite the lack of a functional kinase domain PTK7 still contributes to downstream signaling of Wnt5a through its interaction with LRP6 (low-density lipoprotein receptor-related protein 6) and FZD (frizzled) receptors. PTK7 expression contributes to oncogenic potential in a variety of solid tumors including ovarian, colorectal and breast cancers and neuroblastoma. The cell surface expression and oncogenic function of PTK7 in a variety of tumor types makes it a compelling target for both CAR (chimeric antigen receptor)-T cell and antibody based therapeutic development. The use of cofetuzumab pelidotin, an ADC (antibody-drug conjugate) based on auristatin and targeting PTK7, showed promise in the treatment of ovarian cancer, NSCLC (non-small cell lung cancer) and TNBC (triple-negative breast cancer), showing the potential of targeting PTK7 in oncology.

Application(s)

- Expression of human PTK7 in cells of interest.
- Generate PTK7-expressing cell pools or stable cell lines 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.

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 for up to 12 months from date of receipt. 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 PTK7 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/kill-curve-protocol), for antibiotic selection of transduced cells followed by clonal selection.

Figures and Validation Data

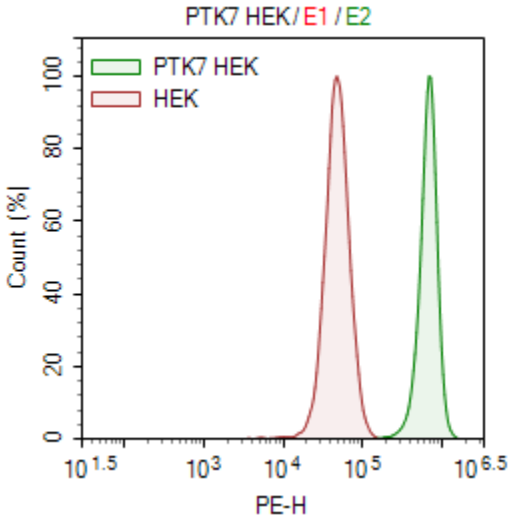

Figure 2. Expression of human PTK7 in HEK293 cells transduced with human PTK7 Lentivirus. Approximately 100,000 HEK293 cells were transduced with 1,000,000 TU of PTK7 Lentivirus. 48 hours post-transduction, cells were stained with PTK7 (CCK-4) Antibody, anti-human, PE (Miltenyi# 130-122-923) and analyzed by flow cytometry. Red, parental HEK293 cells; Green, HEK293 cells transduced with PTK7 lentivirus. Y-axis represents the % cell number. X-axis indicates PE intensity.

Sequence
Human PTK7 sequence (NM_002821.5)

MGAARGSPARPRRLPLLSVLLLPLLGGTQTAIVFIKQPSSQDALQGRRALLRCEVEAPGPVHVYWLLDGAPVQDTERRFAQGSSL
SFAAVDRLQDSGTFQCVARDDVTGEEARSANASFNIKWIEAGPVVLKHPASEAEIQPQTQVTLRCHIDGHPRPTYQWFRDGTPL
SDGQSNHTVSSKERNLTLRPAGPEHSGLYSCCAHSAFGQACSSQNFTLSIADESFARVVLAPQDVVVARYEEAMFHCQFSAQPP
PSLQWLFEDETPITNRSRPPHLRRATVFANGSLLLTQVRPRNAGIYRCIGQGQRGPPIILEATLHLAEIEDMPLFEPRVFTAGSEERV
TCLPPKGLPEPSVWWEHAGVRLPTHGRVYQKGHELVLANIAESDAGVYTCHAANLAGQRRQDVNITVATVPSWLKKPQDSQLE
EGKPGYLDCLTQATPKPTVVWYRNQMLISEDSRFEVFKNGTLRINSVEVYDGTWYRCMSSTPAGSIEAQARVQVLEKLKFTPPPQ
PQQCMEFDKEATVPCSATGREKPTIKWERADGSSLPEWVTDNAGTLHFARVTRDDAGNYTCIASNGPQGQIRAHVQLTVAVFI
TFKVEPERTTVYQGHTALLQCEAQGDPKPLIQWKGKDRILDPTKLGPRMHIFQNGSLVIHDVAPEDSGRYTCIAGNSCNIKHTEA
PLYVVDKPVPEESEGPGSPPPYKMIQTIGLSVGAAVAYIIAVLGLMFYCKKRCKAKRLQKQPEGEEPEMECLNGGPLQNGQPSAEI
QEEVALTSLGSGPAATNKRHSTSDKMHFPRSSLQPITTLGKSEFGEVFLAKAQGLEEGVAETLVLVKSLQSKDEQQQLDFRRELEM
FGKLNHANVVRLLGLCREAEPHYMVLEYVDLGDLKQFLRISKSKDEKLKSQPLSTKQKVALCTQVALGMEHLSNNRFVHKDLAAR
NCLVSAQRQVKVSALGLSKDVYNSEYYHFRQAWVPLRWMSPEAILEGDFSTKSDVWAFGVLMWEVFTHGEMPHGGQADDE
VLADLQAGKARLPQPEGCPSKLYRLMQRCWALSPKDRPSFSEIASALGDSTVDSKP

References
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Jie Y., *et al.,* (2021) *Front. Immunol*. 12:665970.
Lee J.Y., *et al.,* (2023) *Cell Rep Med* 4(6):101091.
Maitland M.L., *et al.,* (2021) *Clin Cancer Res* 27 (16): 4511–4520.

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

Related Products

Products	*Catalog #*	*Size*
PTK7 CHO Cell Line	82627	2 vials

Version 082624