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Zuschläge

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- Trockeneiszuschlag
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Description

Cyno EpCAM 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 transduce cynomolgus monkey (*Macaca fascicularis*) EpCAM (epithelial cell adhesion molecule; XM_045369368.1) driven by an EF1A promoter, and a puromycin selection marker (Figure 1).

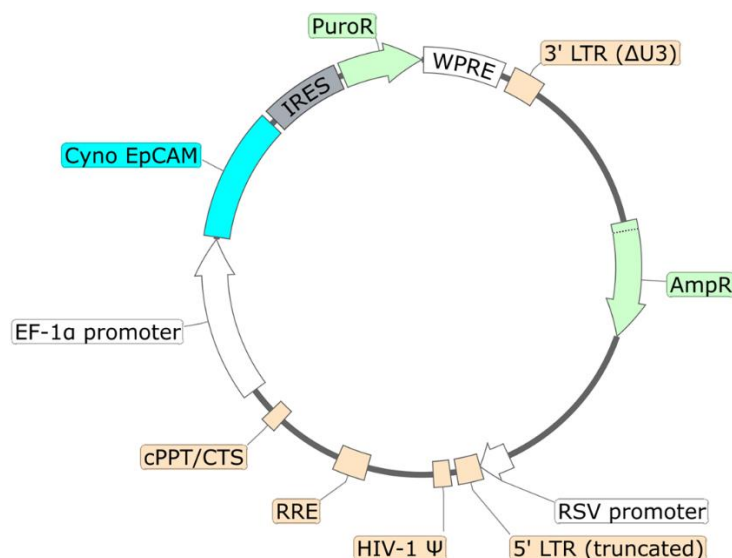


Figure 1. Schematic of the lenti-vector used to generate the Cyno EpCAM Lentivirus.

Background

EpCAM (Epithelial cell adhesion molecule), also known as CD90, is specific to epithelial cells and regulates cell proliferation and differentiation. It is used as an epithelial cell biomarker to detect circulating tumor cells. EpCAM belongs to the small GA733 protein family and is a homophilic transmembrane glycoprotein involved in cell-cell adhesion and tissue plasticity. It is a single-chain membrane-spanning protein with three major domains known as EpEX, involved in epidermal growth factor receptor (EGFR) mediated signaling pathways. Due to the versatile function of EpCAM and its crosstalk with other signaling pathways in cancer, EpCAM is considered an attractive target for cancer diagnosis, prognosis, and therapy.

Application

Generate cell pools or stable cell lines expressing Cyno EpCAM, following puromycin selection.

Formulation

The lentiviruses 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 virus 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 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. 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 Cyno EpCAM 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.

Validation Data

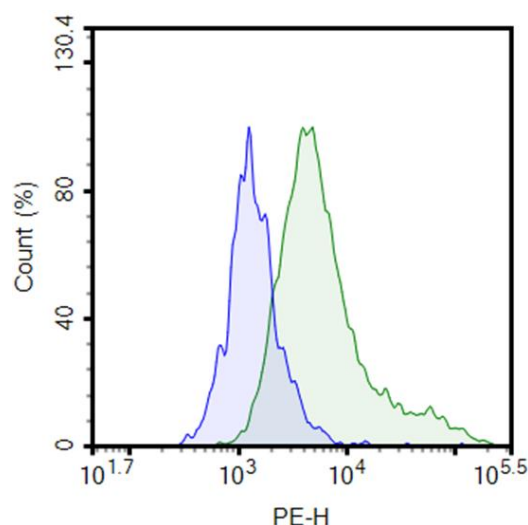


Figure 2. Expression of Cyno EpCAM in CHO-K1 transduced with Cyno EpCAM Lentivirus was analyzed by flow cytometry.

Approximately 50,000 CHO-K1 cells were transduced with 500,000 TU of Cyno EpCAM Lentivirus. 66 hours post-transduction, the cells were selected with 5 µg/ml of puromycin. The puromycin resistant cell pool (green) was stained using PE anti-human CD326 (EpCAM) Antibody (Biolegend #324205) and analyzed by flow cytometry. Non-transduced CHO-K1 cells were run in parallel as control (blue). The y axis represents the % of cells. The x axis indicates fluorophore intensity.

Sequence

Cyno EpCAM (XM_045369368.1)

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MAPPQVLAFGLLLAAATASFAAAQKEVCENYKLAVNCFLNDNGQCQCTSIGAQNTVLCSKLAACKCLVMKAEMNGSKLGRRAK
PEGALQNNDGLYDPDCDESGLFKAKQCNGTSTCWCVNTAGVRRTDKDEITCSERVRTYWIIIELKHKAREKPYDVQSLRTALEE
AIKTRYQLDPKFITNILEYEDNVITIDLQVNSSQKTQNDVDIADVAYYFEKDVKGESLFHSHKMDLRVNGEQLDLDPGQTLIYYVDEK
APEFSMQGLKAGVIAVIVVVVIAIVAGIVVLVISRKKRMAKYEKAEIKEMGEIHRELNA
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Troubleshooting Guide

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

References

Liu Y., *et al.*, 2022, *Exp Hematol Oncol* 11: 97.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
EpCAM CHO Cell Line	78683	2 vials
EPCAM, Avi-His-Tag Recombinant	100461	25 µg/100 µg
EPCAM, Avi-His-Tag, Biotin Labeled Recombinant	100462	25 µg/50 µg
Human EpCAM Lentivirus	78718	500 µl x 2

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