Description

The EpCAM Lentiviruses 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. The particles contain a human EpCAM (NM_002354.3) driven by an EF1A promoter and a puromycin selection marker (Figure 1).

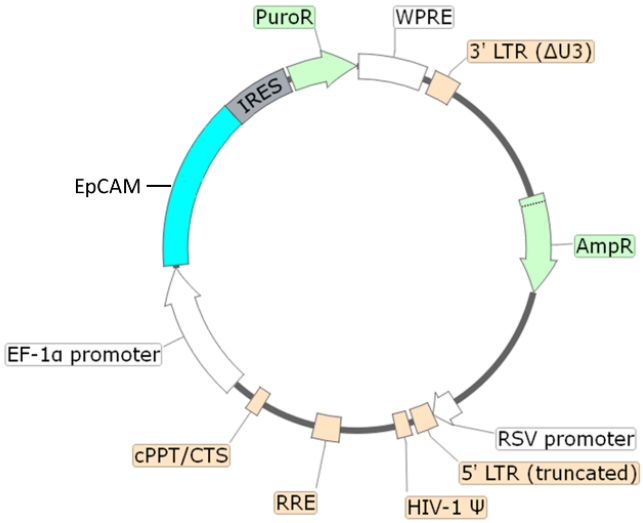

Figure 1: Schematic of the lenti-vector used to generate the EpCAM Lentivirus

Background

Epithelial cell adhesion molecule (EpCAM, 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(s)

Generation of a stable cell line expressing human EpCAM with puromycin selection

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

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 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 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 EpCAM 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) for antibiotic selection of transduced cells. Visit: https://bpsbioscience.com/cell-line-faq for guidelines on performing a kill curve.

Figures and Validation Data

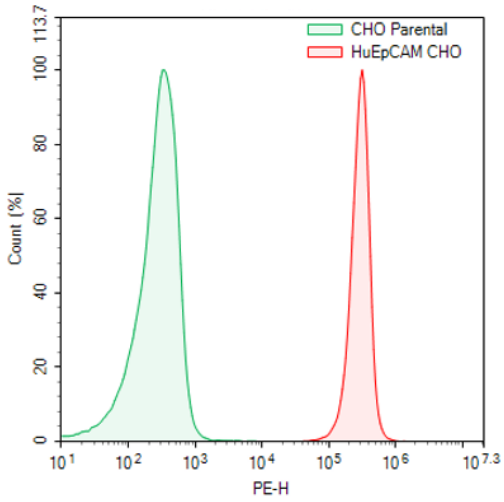

Figure 2. Transduction of CHO-K1 using EpCAM Lentivirus
Approximately 50,000 CHO-K1 cells were transduced with 500,000 TU of EpCAM lentivirus. After 66 hours of transduction, the cells were selected with 5 µg/ml of puromycin. The puromycin-resistant cell pool was stained using PE anti-human EpCAM Antibody (Biolegend #324205) and analyzed by flow cytometry.

Sequence

Human EpCAM (NM_002354.3)
MAPPQVLAFGLLLAAATATFAAAQEECVCENYKLAVNCFVNNNRQCQCTSVGAQNTVICSKLAAKCLVMKAEMNGSKLGRRA
KPEGALQNNDGLYDPDCDESGLFKAKQCNGTSMCWCVNTAGVRRTDKDTEITCSERVRTYWIIIELKHKAREKPYDSKSLRTALQ
KEITTRYQLDPKFITSILYENNVITIDLVQNSSQKTQNDVDIADVAYYFEKDVKGESLFHSKKMDLTVNGEQLDLDPGQTLIYYVDEK
APEFSMQGLKAGVIAVIVVVVIAVVAGIVVLVISRKKRMAKYEKAEIKEMGEMHRELNA

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*
EpCAM CHO Cell Line	78683	2 vials
EPCAM, Avi-His-Tag Recombinant	100461	100 µg
EPCAM, Avi-His-Tag, Biotin Labeled Recombinant	100462	25 µg, 50 µg