

Description

The CD20 Lentiviruses are replication incompetent, HIV based, VSV-G pseudotyped lentiviral particles that are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. These viruses constitutively express human CD20 (NM_021950) under the control of an EF1A promoter (Figure 1).

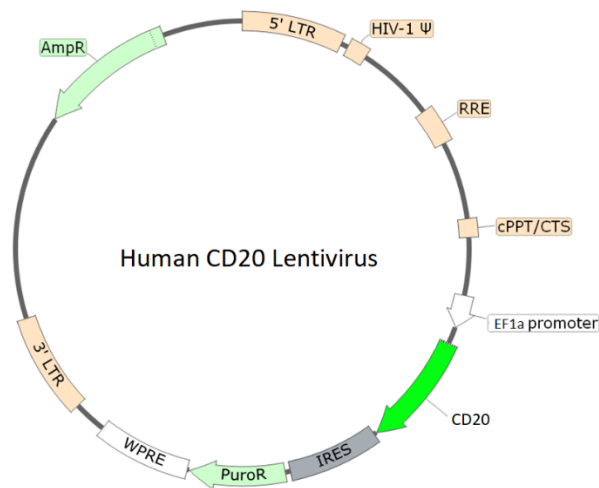


Figure 1. Schematic of the lenti-vector used to generate the CD20 Lentivirus.

Background

CD20 (also known as membrane spanning 4-domains A1 or MS4A1) is a glycosylated phosphoprotein expressed on the cell surface of B cells. Although the functional significance of CD20 is not clear, it has been shown to regulate intracellular calcium levels. CD20 is a highly attractive target antigen for immunotherapy because it is expressed in more than 90% of patients with B-cell lymphoma. First approved in 1997, Rituximab (Rituxan) is a chimeric monoclonal antibody targeting CD20 and has been classified by the World Health Organization as an “Essential Medicine”. Since then, additional monoclonal antibodies against CD20 have been approved or are being tested in clinical trials for the treatment of multiple sclerosis (MS), chronic lymphocytic leukemia (CLL), follicular lymphoma, diffuse large B cell lymphoma (DLBCL), rheumatoid arthritis, non-Hodgkin’s lymphoma, systemic lupus erythematosus, and myalgic encephalomyelitis (chronic fatigue syndrome). More recently, anti-CD20-CD19 bispecific CAR-T cells have been developed.

Application

- Study the transient expression of human CD20 in target cells.
- Generate a stable cell line expressing human CD20 with limiting dilution under 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 μ l x 2) of lentivirus at a titer $\geq 10^7$ TU/ml. The titer will vary with each lot; the exact value will be 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

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 CD20 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 antibiotics selection of transduced cells.

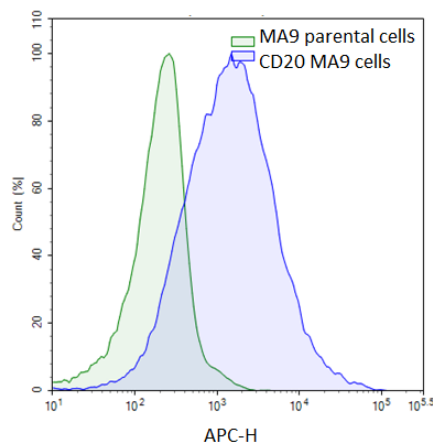
Figures and Validation Data

Figure 2. Transduction of MA9 cells using the CD20 lentivirus.

Approximately 50,000 MA9 cells were transduced with 500,000 TU of CD20 lentivirus via spinoculation (32°C x 30 min in the presence of $5\ \mu\text{g}/\text{ml}$ of polybrene). After 48 hours of transduction, the cells were selected with $0.5\ \mu\text{g}/\text{ml}$ of puromycin. The puromycin resistant cell pool was stained with APC-labeled anti-human CD20 (clone#2H7; Biolegend #302310) and analyzed by flow cytometry.

Troubleshooting Guide

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

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
CD19 Lentivirus	78657	500 µl x 2
CD22 Lentivirus	78659	500 µl x 2