Description

The untransduced T cells are produced by mock lentiviral transduction of human primary CD4+CD8+ T cells. These cells are subjected to comparable manipulations as CAR-T cells: activation, spinoculation (without lentivirus), and expansion. These T cells are meant to be negative controls in experiments using lentivirus-transduced primary CAR-T cells.

Application

Negative control for lentivirus-transduced CAR-T cells

Materials Provided

Components	Format
One vial of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of CryoStor® CS10)

Mycoplasma Testing

The cells have been screened to confirm the absence of Mycoplasma species.

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Recommended T Cell Medium: StemSpan SFEM (Stemcell Technologies, #09650) supplemented with 10% heat-inactivated FBS (Life Technologies, #10082147), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01), plus 10 ng/ml IL-2 (BPS Bioscience, #90184)

Cell Thawing and Culture Protocol:

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed T cell growth medium.

   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed T cell growth medium.
3. Transfer the resuspended cells to a T25 flask and add T cell activation reagents. Activate the cells at 37°C with 5% CO_2 for 24 – 48 hours.
4. Centrifuged the cells gently at 300 x g for 5 minutes and resuspend in fresh T cell medium. Continue to culture the cells at 37°C with 5% CO_2. Do not allow the cell density to exceed 2.0 x 10^6 cells/ml. Transfer the cells in larger culture vessels and add fresh medium when the density reaches 2.0 x 10^6 cells.

It is recommended to activate the untransduced T cells for expansion after thawing. Since these are primary cells, the extent of expansion is not predictable. Perform the cytotoxicity assay as soon as possible to avoid exhaustion. The untransduced T cells should not be in culture for more than 8-10 days. It is not recommended to freeze the cells again once they have been activated and expanded.

Validation

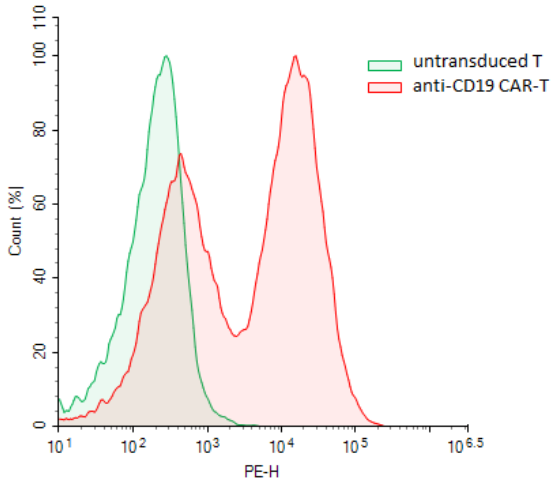

Figure 2: The expression of anti-CD19 CAR in anti-CD19 CAR-T cells, with untransduced T cells as negative control. Anti-CD19 CAR-T cells (BPS Bioscience #78171) and untransduced T cells were thawed and activated for 48 hours. Anti-CD19 CAR-T and untransduced T cells were then expanded for another 4 days, and ~50,000 cells were analyzed by flow cytometry using PE-anti-FMC63 ScFv (Acrobiosystems, #FM3-HPY53-25tests). Untransduced T cells were used as negative control.

Warnings

Donors have been screened and determined negative for:

- Hepatitis B (anti-HBc EIA, HBsAg EIA)
- Hepatitis C (anti-HCV EIA)
- Human Immunodeficiency Virus (HIV-1/HIV-2 plus O)
- Human T-Lymphotropic Virus (HTLV-I/II)
- HIV-1/HCV/HBV
- West Nile Virus
- Trypanasoma cruzi

Note: *Testing cannot guarantee that any sample is completely virus-free. These cells should be treated as potentially infectious and appropriate biological safety level 2 precautions should be used.*

Troubleshooting Guide

For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Anti-CD19 CAR-T Cells	78171	1 vial
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ)	78600	50 µl
Anti-BCMA CAR Lentivirus (Clone C11D5.3 ScFv-CD8-CD28-CD3ζ)	78603	50 µl
Anti-CD20 CAR Lentivirus (Clone Leu-16 ScFv-CD8-4-1BB-CD3ζ)	78606	50 µl
Anti-CD22 CAR Lentivirus (Clone m971 ScFv-CD8-4-1BB-CD3ζ)	78608	50 µl
Anti-CD19/CD22 Bispecific CAR Lentivirus (Clones FMC63/m971 ScFv-CD8-4-1BB-CD3ζ)	78609	50 µl