Description

Recombinant HEK293 cell line constitutively expressing human TNFR2 gene (TNFRSF1B or CD120b, NM_001066.3) under the control of the CMV promoter.

Background

Tumor Necrosis Factor Receptor 2 (TNFR2, also known as TNFRSF1B or CD120b) is a transmembrane receptor of the TNF protein superfamily that binds the pleiotropic pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha). TNFR2 can be found in several T-Cell subsets such as regulatory $CD8^+$ T-Cells (Tregs) and $CD4^+$ tumor-infiltrating T cells, myeloid lineage cells and some cancer types, and it is involved in autoimmune diseases, graft versus host disease and cancer. It has become an attractive target for cancer immunotherapy, where its different functions as oncogene and immune regulator are being explored. TNFR2 also exhibits neuroprotective properties and promotes tissue regeneration, making it a promising potential therapeutic target for the treatment of Alzheimer's disease.

Application

- Screen and characterize TNFR2 antibodies.
- Study TNFR2 signaling in HEK293 cells.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1N	BPS Bioscience #79801

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.

Media Formulations

For best results, the use of validated and optimized media by BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1N (BPS Bioscience #79801):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 0.5 µg/mL Puromycin.

Cell Culture Protocol

Cell Thawing

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 Thaw Medium 1 **(no Puromycin)**.
   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 Thaw Medium 1 **(no Puromycin)**.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 **(no Puromycin)** and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. For the first passage and subsequent passages, use Growth Medium **1N (contains Puromycin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium **1N** and transfer to a tube. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1N **(contains Puromycin)**. Seed into new culture vessels at the recommended sub-cultivation ratio range of 1:8 to 1:10 weekly or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1N and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

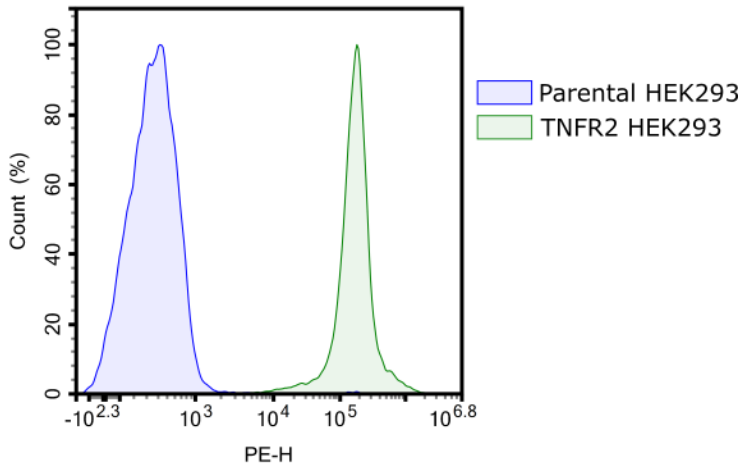

Figure 1: Expression of TNFR2 in TNFR2 HEK293 Cell Line.
TNFR2 HEK293 cells (green) or control parental HEK293 cells (blue) were stained with PE-conjugated Anti-TNFR2 Antibody (Biolegend #358404) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

Sequence
Human TNFR2 sequence (accession number NM_001066.3)

MAPVAVWAALAVGLELWAAAHALPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDST
YTQLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCRPGFGVARPGTETSDVVCKPCA
PGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSPTRSMAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPP
AEGSTGDFALPVGLIVGVTALGLLIIGVVNCVIMTQVKKKPLCLQREAKVPHLPADKARGTQGPEQQHLLITAPSSSSSSLESSASAL
DRRAPTRNQPQAPGVEASGAGEARASTGSSDSSPGGHGTQVNVTCIVNVCSSSDHSSQCSSQASSTMGDTDSSPSESPKDEQV
PFSKEECAFRSQLETPETLLGSTEEKPLPLGVPDAGMKPS*

References

1. Chen X, *et al*. (2017). *Science Signaling*, 10(462): 2328
2. Chopra M, *et al.* (2016) Exogenous TNFR2 activation protects from acute GvHD via host T reg cell expansion. *Journal of Experimental Medicine* 213: 1881-1900
3. Medler J, *et al.* (2022) Tumor Necrosis Factor Receptor 2 (TNFR2): An Emerging Target in Cancer Therapy. *Cancers* 14(11): 2603
4. Vanamee É. and Faustman D. (2017). TNFR2: A Novel Target for Cancer Immunotherapy. *Trends in Molecular Medicine*, 23(11): 1037-1046

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Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
TNFR2, Fc-Fusion (IgG1), His-Avi-Tag, Biotin-labeled Recombinant	100205	25 µg/50 µg
TNFR2:TNF-alpha[Biotinylated] Inhibitor Screening Assay	79756	96 reactions
TNFR2, Fc-Fusion (IgG1), His-Avi-Tag Recombinant	79363	100 µg
Human Tumor Necrosis Factor-alpha Recombinant	90244-B	50 µg