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Description

Recombinant clonal HEK293 stable cell line constitutively expressing full length human Transferrin Receptor Protein-1 (TFRC and TFR1, also known as human CD71 protein, GenBank accession #NM_001128148). The surface expression of TFRC was confirmed by flow cytometry.

Background

Transferrin Receptor Protein-1 (TFRC) is a cell surface receptor that binds ferric-iron-loaded transferrin in the bloodstream at high affinity to facilitate iron uptake into cells. TFRC is therefore an integral component of the body's iron supply chain and general homeostasis. Intracellular iron levels, hypoxia and CRE signaling regulate TFRC transcription. In addition, TFRC is upregulated in correlation with tumor-progressive stages of multiple forms of cancer. At the cellular level iron surplus results in oxygen radical formation and cellular dysfunction, while iron deficiency can cause rapid cell death. Deregulated iron levels are therefore correlated with multiple disease states including Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease, Multiple Sclerosis (MS), and tumorigenesis.

Equally important are potential therapeutic uses of TFRC. As TFRC is upregulated on the surface of tumor cells, it has been targeted to inhibit adult T-cell leukemia/lymphoma. Additionally, TFRC has found important utility in treatments of neurological diseases. Drug discovery in the central nervous system (CNS) space has historically been challenged by limited access of therapeutics across the blood brain barrier (BBB). As TFRC is highly expressed on the surface of brain capillary endothelial cells within the BBB, drug delivery strategies are being developed to leverage transcytosis of TFRC-bound entities from the bloodstream to the CNS (i.e. receptor mediated transport, or RMT). Current approaches include PEGylated liposomes coated with the TFRC ligand transferrin, as well as anti-TFRC antibodies linked to nanoparticles (immunoliposomes) carrying a therapeutic payload.

Application

1. Useful for screening or performing binding assays of antibodies designed against human Transferrin Receptor-1 in a cellular context.
2. Study of endocytosis kinetics on transferrins and their derivatives in cellular uptake assays for the development of next generation culture media formulations.
3. Preliminary assessment of transferrin receptor-targeted nanoparticle complexes (immunoliposomes) designed to deliver therapeutic payloads across the blood brain barrier (BBB) via transcytosis.

Materials Provided

Components	Format
2 vials of frozen cells	~2 x 10 ⁶ cells in 1 ml of 10% DMSO

Host Cell

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

Mycoplasma Testing

The cell line has been screened using the MycoAlert™ Mycoplasma Detection kit (Lonza, #LT07-218) to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems 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.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1C	BPS Bioscience #79532

Storage Conditions

Cells will arrive upon 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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.



Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO₂ using Growth Medium 1C.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187): MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Thermo Fisher, Cat. #26140079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 1C (BPS Bioscience #79532):

Thaw Medium 1 (BPS Bioscience, #60187) plus 50 µg/ml of Hygromycin B (Thermo Fisher, #10687010) to ensure stability of expression.

Assay Medium: Thaw Medium 1 (BPS Bioscience, #60187)

Cell Culture Protocol*Cell Thawing*

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Hygromycin B**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 (**no Hygromycin B**).
3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO₂ incubator.
4. After 24 hours of culture, add an additional ~3 ml of Thaw Medium 1 (**no Hygromycin B**), and continue growing culture in a CO₂ incubator at 37°C until the cells are ready to be split.

- Cells should be split before they are fully confluent. At first passage, switch to Growth Medium 1C (contains Hygromycin B).

Cell Passage

- To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.25% Trypsin/EDTA.
- After detachment, add Growth Medium 1C (contains Hygromycin B) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1C and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: about 1:5 every 5 days.

Cell Freezing

- To freeze down the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.25% Trypsin/EDTA.
- After detachment, add Thaw Medium 1 (no Hygromycin B) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience, #79796) at $\sim 2 \times 10^6$ cells/ml.
- Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
- Transfer to liquid nitrogen the next day for storage.



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

Validation Data

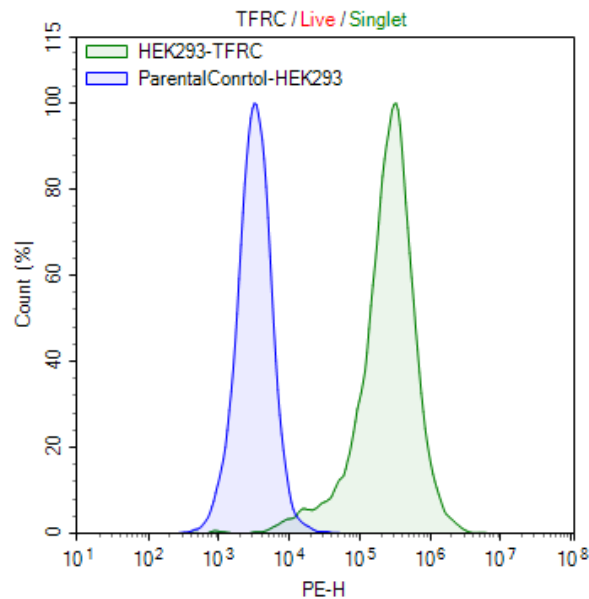


Figure 1. Expression of TFRC validated by flow cytometry.

Flow cytometry using PE-conjugated anti-human TFRC antibody (anti-CD71, BioLegend, #334106) to detect TFRC surface expression on either the TFRC-HEK293 Recombinant Cell Line (green) or parental HEK293 cells (blue).

Sequence

Human TFRC Sequence (Accession Number: NM_001128148)

MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNHVMKLAVDDEENADNNTKANVTKPKRCSGSICYGTIAVIVFFLIGFMIGY
 LGYCKGVEPKTECERLAGTESPVREEPGEDFPAARRLYWDDLKRKLSEKLDSTDFGTIKLLNENSYPREAGSQKDENLALYVEN
 QFREFKLSKVWRDQHFVKIQVKDSAQNSVIIVDKNGRLVYLVENPGGYVAYSKAATVTGKLVHANFGTKKDFEDLYTPVNGSIVI
 VRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIVNAELFFGHHLGTGDPYTPGFPSFNHTQFPSPRSGLPNIPVQTISRAAA
 KLFGNMEGDCPSDWKTDSTCRMVTSESKNVKLTVSNVLKEIKILNIFGVIKGFVEPDHYVVVGAQRDAWGPAAKSGVGTALLL
 KLAQMFSDMVLKDGFPQRSIIIFASWSAGDFGSVGAETEWLEGYLSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQNV
 KHPVTGQFLYQDSNWASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTMTDYTEKELIERIPELNKVARAAAEEVAGQFVIK
 LTHDVELNLDYERYNSQLLSFVRDLNQYRADIKEMGLSLQWLYSARGDFFRATSRLTDFGNAEKTDRFVMKKLNDRVMRVEYH
 FLSPYVSPKESPRHFVWGSHTLPALLENLKLKQNNGAFNETLFRNQLALATWTIQGAANALS GDVWDIDNEF

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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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
HSP90α N-Terminal Domain Assay Kit	50293/50294	96 rxns/384 rxns
Human EGFRvIII – CHO K1 Recombinant Cell Line (High or Low Expression)	78145	2 vials
BCMA CHO Recombinant Cell line (High or Low Expression)	79500	2 vials
CD20 CHO Recombinant Cell Line (High or Medium Expression)	79624	2 vials
HER2 (ERBB2) CHO Recombinant Cell Line (High, Medium or Low Expression)	79612	2 vials
SLAMF7 (CS1) CHO Recombinant Cell Line (High, Medium or Low Expression)	79608	2 vials