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Description

Recombinant HEK293 cells expressing cynomolgus monkey (*Macaca fascicularis*) GPRC5D (the orphan G protein-coupled receptor, class C group 5 member D, accession #XP_005570250.1) under the control of a CMV promoter.

Background

The orphan G protein-coupled receptor, class C group 5 member D (GPRC5D) is a retinoic acid-inducible protein with seven transmembrane segments and a short N-terminal extracellular region. The strong and selective expression of GPRC5D in Multiple Myeloma cells, similar to the expression pattern of B-cell maturation antigen (BCMA), makes it a promising target for the treatment of Multiple Myeloma. It is anticipated that GPRC5D will be an alternative CAR T cell target for Multiple Myeloma immunotherapy.

Application

1. Cynomolgus monkey GPRC5D binding assays; screen for GPRC5D ligands and or antibodies.
2. Target cells for anti-GPRC5D CAR-T development.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2×10^6 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 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 1C	BPS Bioscience #79532

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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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°C 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 1C (BPS Bioscience #79532):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 50 µg/ml of Hygromycin B

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 Hygromycin B**).
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 Hygromycin B**).
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO₂ incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 (**no Hygromycin B**), and continue growing in a 5% CO₂ incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1C (**contains Hygromycin B**).

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 1C 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 1C (**contains Hygromycin B**). Seed into new culture vessels at the desired sub-cultivation ratio of 1:5 weekly.

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 1C and count the cells.
3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10⁶ cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. 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 storage.



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

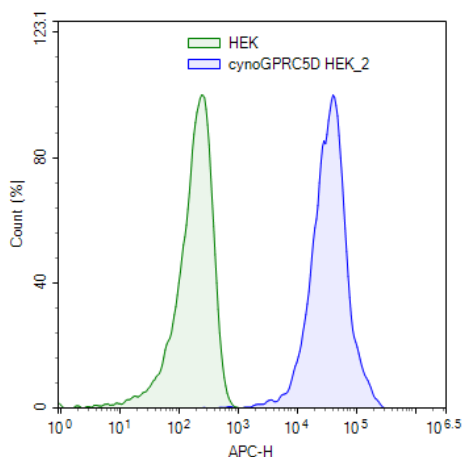
Validation Data

Figure 1. Flow cytometry analysis of cell surface expression of GPRC5D in cynomolgus GPRC5D HEK293 Cell Line. Cynomolgus GPRC5D HEK293 cells (blue) or control HEK293 cells (green) were stained using GPRC5D APC-conjugated Antibody (R&D Systems, #FAB6300A), and the expression of GPRC5D was analyzed by flow cytometry.

Sequence

Cynomolgus monkey (*Macaca fascicularis*) GPRC5D sequence:

MYKDCIESTGDYFLPCDSEGPWGIVLES LA ILGIVVTILLLLAFLFLMRKIQDCSQWNVLP TQLLFLLSVLGLFGLAF AFIIQLNQQTAPVRYFLFGVLFALCFSCLLAHASNLV KLVRGRVSFSWTTILCIAIGCSLLQVIAIEYVTLIMTRGMMFVHMTPYQLNVDV FVLLVYV LFLMALTFFVSKATFCGPCENWKQHGR LIFITVLF SIIIWVWVISMILLRGNPQFQRQPQWDDPVVCIALVTNAWVFLLLYIVPELCI LYRSCRQECPSQGHACPV TAYQRSFQVENQELSRDC

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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
GPRC5D (Cynomolgus) CHO Cell Line	78338	2 vials
GPRC5D CHO Cell Line	78337	2 vials
GPRC5D HEK293 Cell Line	78345	2 vials