Description

STEAP1 HEK293 Cell Line is a HEK293 cell line expressing STEAP1 (six transmembrane epithelial antigen of the prostate 1; NM_012449.3) under the control of a cytomegalovirus (CMV) promoter. This cell line was generated by lentiviral transduction followed by puromycin selection and limited dilution. Individual clones were screened for STEAP1 expression levels by flow cytometry, and a clone was selected to generate this cell line.

This cell line has been validated by flow cytometry.

Background

STEAP1 (six transmembrane epithelial antigen of the prostate 1), also called STEAP, belongs to the STEAP family of metalloproteinases, which are involved in iron and copper regulation and other processes. It is a cell surface protein expressed in several types of cancer, most notably prostate cancer where STEAP1 was identified as a cancer specific cell-surface antigen. The upregulation of STEAP1 is linked to promotion of cancer cell proliferation and metastatic transformation and higher STEAP expression levels are correlated with poor survival. The differential expression of STEAP1 between cancer cells and normal tissue makes STEAP1 a promising target for antibody-drug-conjugate (ADC) development. The use of STEAP1 CAR (chimeric antigen receptor)-T cell therapy combined with tumor-localized IL-12 therapy is also under study for the treatment of prostate cancer.

Application

- Screen therapeutic antibodies and ADCs targeting STEAP1
- Co-culture assays with STEAP1-directed CAR-T cells.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 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 from 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 to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 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 0.5µg/ml of Puromycin.

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

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.

   Note: 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.

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, 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. At first passage and subsequent passages, use Growth Medium 1N.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, 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.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1N.

   Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, 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 cells in 4°C Cell 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

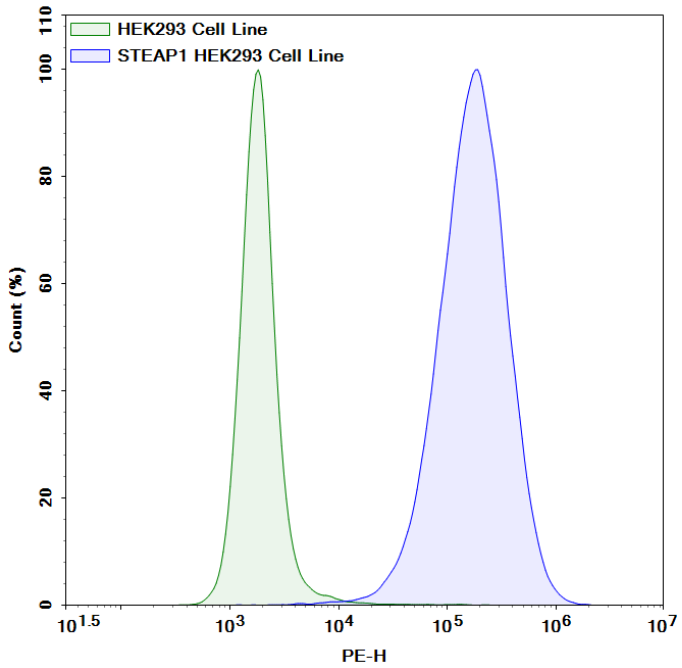

Figure 1: Flow cytometry analysis of STEAP1 cell surface expression in STEAP1 HEK293 Cell Line. STEAP1 HEK293 cell line (green) and control parental HEK293 cells (blue) were stained with FluoSite™ Anti-STEAP1 Antibody, PE-Labeled (#102639) and analyzed by flow cytometry. The y-axis shows the % cell number, and the x-axis characterizes the PE intensity.

Data shown is representative.

Sequence

Human STEAP1 sequence (accession number NM_012449.3)

MESRKDITNQEELWKMKPRRNLEEDDYLHKDTGETSMLKRPVLLHLHQTAHADEFDCPSELQHTQELFPQWHLPIKIAAIIASLT
FLYTLLREVIHPLATSHQQYFYKIPILVINKVLPMVSITLLALVYLPGVIAAIVQLHNGTKYKKFPHWLDKWMLTRKQFGLLSFFFAVL
HAIYSLSYPMRRSYRYKLLNWAYQQVQQNKEDAWIEHDVWRMEIYVSLGIVGLAILALLAVTSIPSVSDSLTWREFHYIQSKLGIV
SLLLGTIHALIFAWNKWIDIKQFVWYTPPTFMIAVFLPIVVLIFKSILFLPCLRKKILKIRHGWEDVTKINKTEICSQL

References

Barroca-Ferreira J., *et al.,* 2018 *Curr Cancer Drug Targets.* 18(3):222-230.
Bhatia V., *et al.,* 2023 *Nat Commun.* 14(1):2041.
Kelly W., *et al.,* 2023 *Cancer Discov.* 14(1):76–89.
Nakamura H., *et al.,* 2023 *Front Oncol.* 13:1285661.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Vandortuzumab STEAP1 Human Antibody	82750	1 mg, 5 mg and 10 mg
FluoSite™ Anti-STEAP1 Antibody, FITC-Labeled	102640	25 tests/ 100 tests

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