

Description

Human EGFRvIII CHO K1 Cell Line (Low Expression) is a CHO K1 cell line stably expressing full-length human EGFRvIII receptor (accession number NP_001333870). Surface expression of hEGFRvIII was confirmed by flow cytometry. The stable clonal cell line was selected for low levels of hEGFRvIII expression to mimic different stages of cancer target cells with low EGFRvIII expression levels.

Background

The epidermal growth factor receptor (EGFR, ErbB-1, HER1) belongs to a family of receptor tyrosine kinases that includes three other closely-related members (HER2, HER3 and HER4). EGFR gene amplification, mutation and re-arrangement are frequently observed in primary glioblastoma multiforme (GBM). The most common EGFR variant in GBM, EGFRvIII, is characterized by a deletion of 267 amino acids in the extracellular domain, leading to a receptor that is unable to bind ligand yet is constitutively active. EGFRvIII enhances the tumorigenic potential of GBM by activating and sustaining mitogenic, anti-apoptotic, and pro-invasive signaling pathways.

Application

Screen for inhibitors of EGFRvIII signaling for immuno-oncology research and drug discovery.

Materials Provided

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

Host Cell

CHO-K1 cell line, Chinese Hamster Ovary, 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 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 3	BPS Bioscience #60186
Growth Medium 3B	BPS Bioscience #79529

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, the use of validated and optimized media from BPS Bioscience is *highly recommended*. 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 to maintain selective pressure on the cell population expressing the gene of interest. Cells should be grown at 37°C with 5% CO₂. BPS Bioscience cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 3 (BPS Bioscience #60186):

F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3B (BPS Bioscience #79529):

F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 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 3.

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 3.
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO₂ incubator.
4. After 48-72 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 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 reach 100% confluency. Switch to Growth Medium 3B at first and subsequent passages.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca²⁺/Mg²⁺, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA following volumes recommended for the cell vessel being used.
2. Once the cells have detached, add Growth Medium 3B 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 3B.
4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:8 weekly or twice per week.

Cell Freezing

1. After detachment, spin down the cells at 300 x g for 5 minutes.
2. Remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at $\sim 2 \times 10^6$ cells/ml.
3. 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.
4. Transfer the vials to liquid nitrogen the next day for long term storage.

Validation

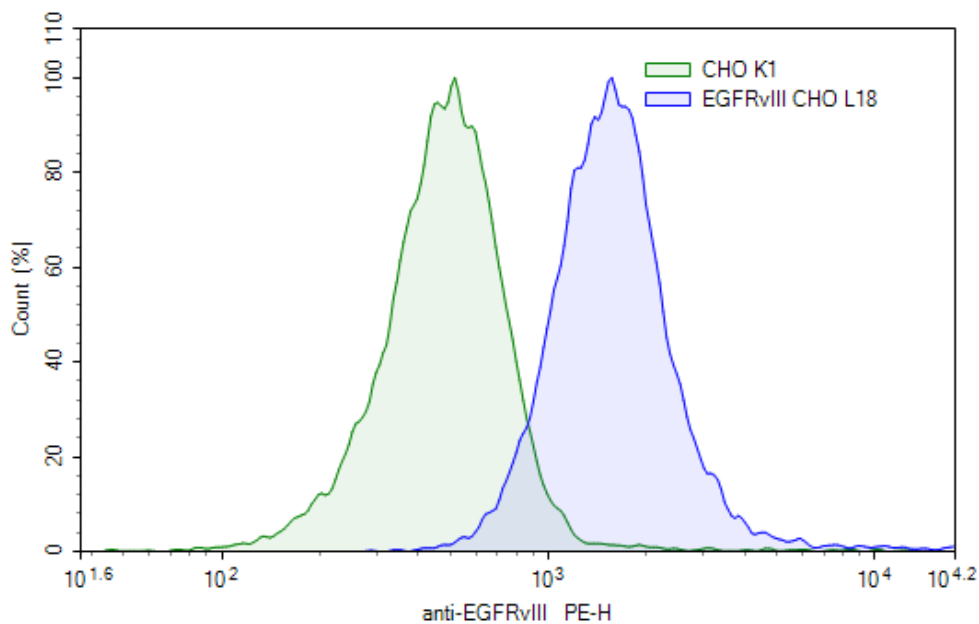


Figure 1. Flow cytometry analysis of cell surface expression of hEGFRvIII in Human EGFRvIII CHO K1 Cell Line (Low Expression).

Human EGFRvIII CHO K1 cells or control CHO-K1 cells were stained with EGFR Antibody (DH8.3) PE-Mutant (NOVUS Biologicals #NBP2-50599PE) and analyzed by flow cytometry. Y-axis represents the % cell number while the X-axis indicates the PE intensity.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence

Human EGFRvIII sequence (accession number: NP_001333870)

MRPSGTAGAA LLALLAALCP ASRALEEKKG NYVVTDHGSC VRACGADSYE MEEDGVRKCK
 KCEGPCRKVC NGIGIGEFKD SLSINATNIK HFKNCTSISG DLHILPVAFR GDSFHTPPL
 DPQELDILKT VKEITGFLLI QAWPENRTDL HAFENLEIIR GRTKQHGQFS LAVVSLNITS
 LGLRSLKEIS DGDVIISGNK NLCYANTINW KKLFGTSGQK TKIISNRGEN SCKATGQVCH
 ALCSPEGCWG PEPRDCVSCR NVSRGRECV D KCNLEGEPR E FVENSECIQ CHPECLPQAM
 NITCTGRGPD NCIQCAHYID GPHCVKTCPA GVMGENNTLV WKYADAGHVC HLCHPNCTYG
 CTGPGLEGCP TNGPKIPSIA TGMV GALLLL LVVALGIGLF MRRRHIVRKR TLRRLLQERE
 LVEPLTPSGE APNQALLRIL KETEFKIKV LGSGAFGTVY KGLWIPEGEK VKIPVAIKEL
 REATSPKANK EILDEAYVMA SVDNPHVCRL LGICLTSTVQ LITQLMPFGC LLDYVREHKD
 NIGSQYLLNW CVQIAKGMNY LEDRRLVHRD LAARNVLVKT PQHVKITDFG LAKLLGAE EK
 EYHAEGGKVP IKWMALESIL HRIYTHQSDV WSYGVTWVWEL MTFGSKPYDG IPASEISSIL
 EKGERLPQP ICTIDVYMIM VKCWMIDADS RPKFRELIIE FSKMARDPQR YLVIQGDERM
 HLPSPDTSNF YRALMDEEDM DDVVDAD EYL IPQQGFFSSP STSRTPLLSS LSATSNNSTV
 ACIDRNLQSQ CPIKEDSFLQ RYSSDPTGAL TEDSIDDTFL PVPEYINQSV PKRPAGSVQN
 PVYHNQPLNP APSRDPHYQD PHSTAVGNPE YLNTVQPTCV NSTFDSPAHW AQKGSHQISL
 DNPDYQQDFF PKEAKPNGIF KGSTAENAEY LRVAPQSSEF IGA

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<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
EGFR Kinase Assay Kit	40321	96 reactions
EGFR(T790M) Kinase Assay Kit	40323	96 reactions
EGFR(L858R) Kinase Assay Kit	40324	96 reactions
EGFR(T790M/L858R) Kinase Assay Kit	40322	96 reactions
EGFR, His-tag, GST-Tag	40187	10 µg
EGFR (T790M), His-tag, GST-Tag	40188	10 µg