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Description

GUCY2C HEK293 Cell Line (Low Expression) is a HEK293 cell line expressing GUCY2C (guanylate cyclase 2C; NM_004963.4) under the control of a cytomegalovirus (CMV) promoter. This cell line was generated by lipophilic transfection followed by hygromycin selection and limited dilution. Individual clones were screened for GUCY2C expression levels by flow cytometry, and a low expressing clone was selected to generate this cell line.

This cell line has been validated by flow cytometry.

Background

Human GUCY2C (Guanylate cyclase 2C) is a membrane-bound receptor primarily expressed in intestinal epithelial cells and, to a lesser extent, in other tissues such as the liver and lung. GUCY2C plays a key role in regulating intestinal fluid and electrolyte homeostasis by catalyzing the conversion of GTP to cyclic GMP (cGMP) upon activation by endogenous ligands such as guanylin and uroguanylin, or by bacterial heat-stable enterotoxins. It is a crucial component of intestinal barrier function and fluid secretion. Dysregulation of GUCY2C signaling has been implicated in gastrointestinal disorders and colorectal cancer. Due to its restricted normal expression and overexpression in certain malignancies, GUCY2C has also emerged as a promising biomarker and therapeutic target for colorectal cancer and other gastrointestinal tumors.

Application

- Screen therapeutic antibodies, ADCs (antibody drug conjugates), or CAR (chimeric antigen receptor) constructs targeting GUCY2C.

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)

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 1F	BPS Bioscience #79540

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% CO₂. 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 1F (BPS Bioscience #79540):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 100 µg/ml Hygromycin B.

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% CO₂ 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% 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 1F.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without $\text{Ca}^{2+}/\text{Mg}^{2+}$, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1F and transfer to a tube.
3. Spin down cells at $300 \times g$ for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1F.
4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:8 twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1F and count the cells.
3. Spin down the cells at $300 \times g$ for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at $\sim 2 \times 10^6$ 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.

Validation Data

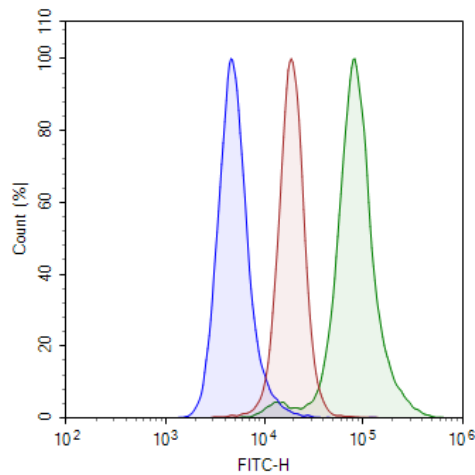


Figure 1: Flow cytometry analysis of GUCY2C cell surface expression in GUCY2C HEK293 Cell Line. High expressing GUCY2C HEK293 cells (#83016-H) (green), low expressing GUCY2C HEK293 cells (#83016-L) (red), and control parental HEK293 cells (blue) were stained with FluoSite™ Anti-GUCY2C Antibody, FITC-Labeled (#102745) and analyzed by flow cytometry. The y-axis shows the % cell number, and the x-axis characterizes the FITC intensity.

Data shown is representative.

Sequence

Human GUCY2C sequence (accession number NM_004963.4)

MKTLLDLALWSLLFQPGWLSFSSQVSQNCHNGSYEISVLMMGNSAFAEPLKNLEDAVNEGLEIVRGRLQNAGLNVTVNATFM
YSDGLIHNSGDCRSSTCEGLDLLRKISNAQRMGCVLIGPSCYSTSTFQMYLDTSELYPMISAGSFGLSFDYKETLRLMSPARKLMYF
LVNFWKTNDLPFKTYSWSTSYVYKNGTETEDCFWYLNALASVSYSFHELGFKVVLRLQDKEFDILMDHNRKSNVIMCGGPEFL
YKLKGDRAVAEDIVILVDFNDQYFEDNVTAPDYMKNVLVLTSPGNSLLNSSFSRNLSPTKRDFALAYLNGILLFGHMLKIFLENG
ENITTPKFAHAFRNLTFEGYDGPVTLDDWGDVDSTMVLLYTSVDTKKYVLLTYDTHVNKTYPVDMSPFTFWKNSKLPNDITGR
GPQILMIAVFTLTGAVVLLLLVALLMLRKYRKDYELRQKKWSHIPPENIFLETNETNHVSLKIDDDKRRDTIQRLRQCKYDKKRVL
KDLKHNDGNFTEKQKIELNKLQIDYYNLTKFYGTVKLDTMIFGVIEYCERGLREVLNDTISYPDGTFMDWEFKISVLYDIAKGMS
YLHSSKTEVHGRKSTNCVVDSDRMVVKITDFGCNSILPPKKDLWTAPEHLRQANISQKGDVYSYGIIAQEILRKETFYTLSCRDRNE
KIFRVENSNGMKPFRPDLFLETAEEKELEVYLLVKNCWEEDPEKRPDFKKIETTLAKIFGLFHDQKNESYMDTLIRRLQLYSRNLHL
VEERTQLYKAERDRADRLNFMPLPRLVVKSLKEKGFVEPELYEEVTIYFSDIVGFTTICKYSTPMEVVDMLNDIYKSFHDHVDHHDV
YKVTETIGDAYMVASGLPKRNGNRHAIDIAKMALEILSFMGTFEHLPLGLPIWIRIGVHSGPCAAGVVGIMPRYCLFGDVTNTAS
RMESTGLPLRIHVSGSTIAILKRTECQFLYEVVRGETYKGRGNETTYWLTGMKDQKFNLPPTVENQQRLQAEFSDMIANSLQK
RQAAGIRSQKPRRVASYKKGTLEYLQLNTTDEKSTYF

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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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
FluoSite™ Anti-GUCY2C Antibody, PE-Labeled	102744	25 tests/ 100 tests
FluoSite™ Anti-GUCY2C Antibody, FITC-Labeled	102745	25 tests/ 100 tests

Version 081325