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Description

Recombinant clonal CHO-K1 stable cell line constitutively expressing full-length human Glycophorin A (GYPA, transcript variant 1, Seq ID: NM_002099.8). The surface expression of Glycophorin A was validated by flow cytometry.

Background

Glycophorin A (GYPA, also known as CD235a) is a sialoglycoprotein and a major intrinsic membrane protein on the surface of human erythrocytes. GYPA plays an important role in the prevention of red cell aggregation in the circulatory system. The Glycophorin A gene contains some antigenic alleles of the MNS blood grouping system for which 40 known variants exist. Several of these antigenic variants have implications for pathogen interaction. For example, the Wright b antigen in the helical region of Glycophorin A acts as a receptor for the malaria parasite *Plasmodium falciparum*. Other variations such as the Mur phenotype causes hemolytic transfusion reaction (HTR) and hemolytic disease in the newborn fetus (HDFN). Glycophorin A is one of the most abundant integral proteins of the red cell membrane, and its genetic sequence varies within a population; therefore, it may also support applications in forensic science.

References

Hassan, S.N. *et al. Transfusion Medicine Review* 2019; 33: 118-124
Ridgewell K. *et al. Biochemical Journal* 1983; 209: 273-276

Application

- Development and optimization of Glycophorin A-binding antibodies or related peptides
- Assay Development

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

CHO-K1 cells, 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 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 3	BPS Bioscience #60186
Growth Medium 3D	BPS Bioscience #79539

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 3 (BPS Bioscience #60186):
Ham's F-12 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3D (BPS Bioscience #79539):
Ham's F-12 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin.

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 **(no Geneticin)**.
   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 **(no Geneticin)**.
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 3 **(no Geneticin)**, 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 3D **(contains Geneticin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3D 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 3D **(contains Geneticin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:10 twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3D 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 10^6 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.

A. Validation Data

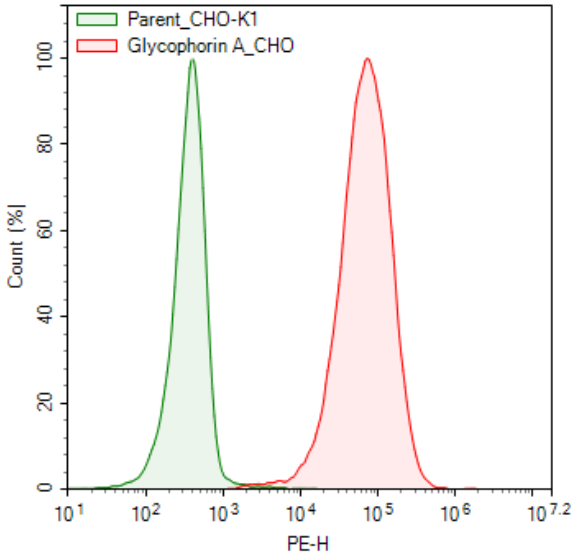

Figure 1: Cell surface expression of Glycophorin A. Flow cytometry validation of Glycophorin A (CD235a) expression on the surface of Glycophorin A (CD235a) CHO cells, represented by a normalized histogram (red) with expression levels compared to control CHO-K1 parental cells (green). Relative phycoerythrin (PE) fluorescence was measured using PE-conjgated antibody (BioLegend #125606) staining.

Sequence
Human glycophorin-A isoform 1 precursor (accession number NP_002090.4)

MYGKIIFVLLLSEIVSISALSTTEVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEP
EITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ

License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

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*
CD235a, Fc Fusion (lgG1), Avi-Tag HiP™	101196	Various sizes
Anti-CD235a IgG1 Antibody, Biotin-Labeled	101098	50 µg
Thaw Medium 3	60186	Various sizes
Growth Medium 3D	79539	500 ml