

Description

Recombinant clonal CHO stable cell line constitutively expressing full length human CD34 protein. The surface expression of CD34 in this cell line was validated by flow cytometry.

Background

CD34 (Cluster of Differentiation-34) is a single-pass transmembrane phosphoglycoprotein and a stem cell antigen. It is a receptor for L-Selectin (CD62L) and CrkL ligands, as well as E- and P-Selectin in the bone marrow extracellular matrix. It can act both as a cell adhesion molecule and as an adhesion blocking molecule against mast cells, dendritic precursors and eosinophils. CD34 is most commonly characterized by its expression on the surface of hematopoietic stem cells (HSC), and is therefore utilized for positive selection and counting of CD34⁺ cells in clinical bone marrow transplants. CD34⁽⁻⁾ HSCs have also been identified, which exhibit a distinct progenitor profile. CD34 bears a controversial identity as a mesenchymal stromal cell (MSC) marker, the evidence for which is highly context-dependent. In addition, CD34 is expressed by other progenitor cell types such as quiescent keratocytes, stem cells within the hair follicle that replace epithelial layers of the skin, and within sub-populations of interstitial cells of Cajal (ICC). More recently CD34 has been identified as a potential predictor of neoadjuvant chemotherapy efficacy in cervical cancer patients, and when found in tumor stroma, is associated with better colorectal cancer survival.

Application

1. Useful for screening or performing binding assays of antibodies recognizing human CD34 in a cellular context.
2. Assessment of reagents designed to characterize the lineage/identity of hematopoietic stem and other progenitor cells.
3. Development of stem cell characterization standards and procedures that include CD34[±] identification.

Materials Provided

Components	Format
2 vials of frozen cells	2 x 10 ⁶ cells in 1 ml of 10% DMSO in FBS

Host Cell

CHO-K1 cells, Chinese Hamster Ovary, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened using the MycoAlert™ Mycoplasma Detection kit (Lonza, #LT07-218) 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 3D	BPS Bioscience #79539

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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS-validated media, formulation can be found below.



Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO₂ using Growth Medium 3D.

Media Required for Cell Culture

Thaw Medium 3 (BPS Bioscience #60186): F12-K (Hyclone #SH30526.01) supplemented with 10% FBS (Thermo Fisher, Cat. #26140079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01)

Growth Medium 3D (BPS Bioscience #79539): F12-K (Hyclone #SH30526.01) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01), plus 1 mg/ml Geneticin (Thermo Fisher, #11811031)

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 3 (**without Geneticin-G418**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 3 (**without Geneticin-G418**).
3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO₂ incubator.
4. After 24 hours of culture, add an additional ~3 ml of Thaw Medium 3 (**no Geneticin-G418**), and continue growing culture in a CO₂ incubator at 37°C until the cells are ready to be split.
5. Cells should be split before they are fully confluent. At first passage, switch to Growth Medium 3D (**contains Geneticin-G418**).

Cell Passage

1. To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.25% Trypsin/EDTA.
2. After detachment, add Growth Medium 3D (**contains Geneticin-G418**) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 3D and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio is about 1:8 every 3 days.

Cell Freezing

1. To freeze down the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.25% Trypsin/EDTA.
2. After detachment, add Thaw Medium 3 (**no Geneticin-G418**) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience, #79796) at $\sim 2 \times 10^6$ cells/ml.
3. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for storage.



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

Validation Data

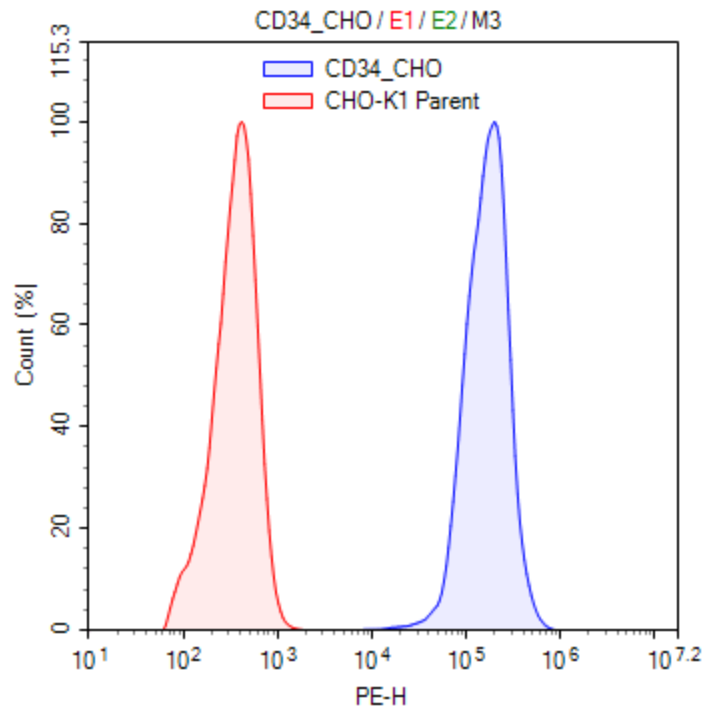


Figure 1. Confirmation of CD34 expression on CHO-K1 recombinant cells

Flow cytometry using PE-conjugated anti-human CD34 antibody (BioLegend, #343505) to detect CD34 surface expression on either the CHO-K1 Recombinant Cell Line (blue) or parental CHO-K1 cells (red).

Sequence

Human CD34 Sequence (Accession Number: NM_001025109)

MLVRRGARAGPRMPRGWTALCLLSLLPSGFMSLDNNGTATPELPTQGTFSNVSTNVSQYQETTPSTLGST
 SLHPVSQHGNEATTNITETTVKFTSTSVITSVYGNTNSSVQSQTSVISTVFTTPANVSTPETTLKPSLSPGNVS
 DLSTTSTSLATSPTKPYTSSSPILSDIKAEIKCSGIREVKLTQGICLEQNKTS SCAEFKDRGEGLARVLCGEEQA
 DADAGAQVCSLLLAQSEVRPQCLLLVLANRTEISSKLQLMKKHQSDLKKGILDFTEQDVASHQSYSQKTLIA
 LVTSGALLAVLGITGYFLMNRRSWSPTGERLGEDPYTENGSGGQGYSSGPGTSPEAQGKASVNRGAQENG
 TGQATSRNGHSARQHVVADTEL

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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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
CD34, Avi-His-Tag HiP™	101190	100 µg
Human Stem Cell Factor	90235-B	10 µg
CD38 CHO Recombinant Cell Line (High, Medium or Low Expression)	79615	2 vials
SLAMF7 (CS1) CHO Recombinant Cell Line (High, Medium, or Low Expression)	79608	2 vials
Thaw Medium 3	60186	100 ml/500 ml
Growth Medium 3D	79539	500 ml