Description

Recombinant clonal CHO-K1 stable cell line constitutively expressing full-length human NCAM1 protein (Neural cell adhesion molecule 1, also known as CD56, single-pass type I membrane protein, isoform 1- Seq ID: NM_000615).

The NCAM1 (CD56) CHO cell line is provided as three different stable, clonal cell lines. Each cell line was selected for different levels of NCAM1 expression: low (L), medium (M) or high (H). Variable expression levels are advantageous for validating antibodies or compounds in binding assays. A drug discovery program may find value in owning all three clones with differential expression of NCAM1. The surface expression of NCAM1 in each cell line was validated in parallel by flow cytometry.

Background

NCAM1 (Neural cell adhesion molecule 1), otherwise known as CD56 (Cluster of Differentiation 56), is a cell surface antigen, glycoprotein and member of the immunoglobulin (Ig) superfamily. Transmembrane isoforms of NCAM1 fulfill their functionality through cell-cell and cell-matrix interactions via homophilic binding (*in cis* and *in trans*), as well as via heterophilic binding to extracellular matrix factors such as fibroblast growth factor receptors and N-cadherin. Each of these interactions elicit intracellular signaling cascades toward effector function.

NCAM1 is prominently known for its roles in nervous system development; specifically, neurite outgrowth, cell migration and synaptic plasticity. Decreased NCAM1 levels in serum are associated with cognitive impairment in vascular dementia. NCAM1 is upregulated in ischemic cardiomyopathy (ICM) models. Importantly, NCAM1 supports immune surveillance through expansion of T and B lymphocyte and natural killer (NK) cells. NCAM1/CD56 is the archetypal phenotypic marker for NK cells, and has also been found on subsets of $CD4^+$ and $CD8^+$ T cells. Deficiencies in $CD56^+$ immune cells have been associated with specific infections and autoimmune diseases. NCAM1 expression is altered in hematological malignancies, such as multiple myeloma and leukemia, and solid tumors from various tissue types. It has been suggested that NCAM1 expression in acute myeloid leukemia (AML) promotes chemotherapy resistance, and therefore may guide alternative treatment decisions in patients.

Application

Development and optimization of NCAM1/CD56-binding antibody or peptide.

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):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3D (BPS Bioscience #79539):
F-12K 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

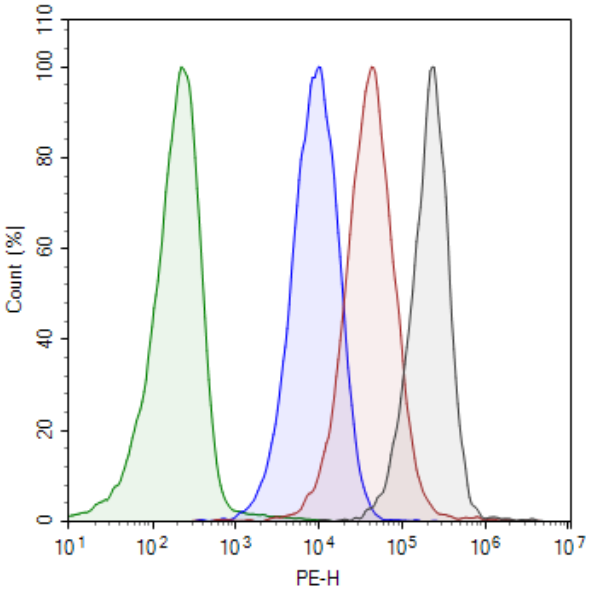

Figure 1: Flow cytometry validation of NCAM1/CD56 expression on the surface of NCAM1/CD56-CHO cells. NCAM1 expression is represented by a normalized histogram with low (blue), medium (red), and high (black) expression levels compared to background control CHO-K1 parental cells (green). Relative phycoerythrin (PE) fluorescence was measured using PE-conjugated antibody (BioLegend #362524) staining of live cells in suspension.

Sequence

>NP_000606.3 Human Neural cell adhesion molecule 1 isoform 1 precursor

MLQTKDLIWTLFFLGTAVSLQVDIVPSQGEISVGESKFFLCQVAGDAKDKDISWFSPNGEKLTPNQQRISVVWNDDSSSTLTIYNA
NIDDAGIYKCVVTGEDGSESEATVNVKIFQKLMFKNAPTPQEFREGEDAVIVCDVVSSLPPTIIWKHKGRDVILKKDVRFIVLSNNY
LQIRGIKKTDEGTYRCEGRILARGEINFKDIQVIVNVPPTIQARQNIVNATANLGQSVTLVCDAEGFPEPTMSWTKDGEQIEQEED
DEKYIFSDDSSQLTIKKVDKNDEAEYICIAENKAGEQDATIHLKVFAKPKITYVENQTAMELEEQVTLTCEASGDPIPSITWRTSTRNI
SSEEKTLDGHMVVRSHARVSSLTLKSIQYTDAGEYICTASNTIGQDSQSMYLEVQYAPKLQGPVAVYTWEGNQVNITCEVFAYPS
ATISWFRDGQLLPSSNYSNIKIYNTPSASYLEVTPDSENDFGNYNCTAVNRIGQESLEFILVQADTPSSPSIDQVEPYSSTAQVQFDE
PEATGGVPILKYKAEWRAVGEEVWHSKWYDAKEASMEGIVTIVGLKPETTYAVRLAALNGKGLGEISAASEFKTQPVQGEPSAPK
LEGQMGEDGNSIKVNLIKQDDGGSPIRHYLVRYRALSSEWKPEIRLPSGSDHVMLKSLDWNAEYEVYVVAENQQGKSKAAHFVF
RTSAQPTAIPANGSPTSGLSTGAIVGILIVIFVLLLVVVDITCYFLNKCGLFMCIAVNLCGKAGPGAKGKDMEEGKAAFSKDESKEPI
VEVRTEEERTPNHDGGKHTEPNETTPLTEPEKGPVEAKPECQETETKPAPAEVKTVPNDATQTKENESKA

References

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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

Products	*Catalog #*	*Size*
NCAM1, Avi-His-Tag, HiP™	101043	100 µg/1 mg
NCAM1, Avi-His-Tag, Biotin-Labeled	101044	25 µg/50 µg
Anti-NCAM1 (CD56) IgG Antibody, Biotin-labeled	101112	100 µg