Description

NKp46 CHO Cell Line is a clonal CHO cell line stably expressing full-length human NKp46 (Natural cytotoxicity triggering receptor 1) (NM_004829). Surface expression of NKp46 was confirmed by flow cytometry. Stable clonal cell lines were selected for different levels of NKp46 expression (low, medium, and high) compared to the parental CHO-K1 cell line.

Background

NKp46 (also known as Natural cytotoxicity triggering receptor 1, or NCR1) is an important natural killer (NK) cell-activating receptor expressed on the surface of human NK cells, and it is involved in cytotoxicity. NKp46 participates in the activation of NK cells against pathogens, tumor cells, and virally infected cells. It also plays an important role in autoimmune conditions, including type I diabetes. NKp46 expression is often conserved on infiltrating NK cells in most solid tumors, and NKp46 is a diagnostic biomarker and possible therapeutic target for gastrointestinal T-cell lymphoproliferative diseases. Although a few infectious NKp46 ligands have been identified, endogenous NKp46 cell surface ligands are unknown. Recently, it was reported that NKp46 binds to externalized calreticulin (ecto-CRT), which translocate from the ER (endoplasmic reticulum) to the plasma membrane in situations of cellular stress such as chemotherapy and senescence. This leads to NK cell degranulation and target cell killing and can participate in controlling cancer. Further studies will elucidate the exact mechanism and roles of NKp46 and its ligands in disease and allow the development of new therapeutics for cancer therapy.

Application(s)

- Screen and validate antibodies against NKp46 for drug discovery and research.
- Perform binding assays to screen for potential NKp46 ligands.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ≥1 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 3J	BPS Bioscience #79974

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℃ with 5% CO_2. BPS Bioscience's 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 3J (BPS Bioscience #79974):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 5 µg/ml of Puromycin.

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% CO_2 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% CO_2 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 3J at first and subsequent passages.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, 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 3J 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 3J.

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 ~2 x 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.

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

A. Validation Data

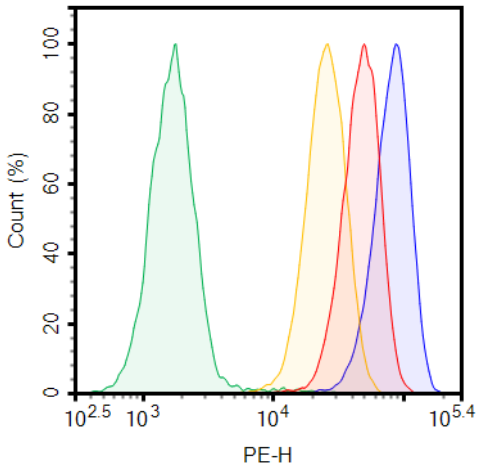

Figure 1: Cell surface expression of NKp46 in NKp46 CHO Cell Line.
NKp46 CHO cell lines and parental CHO-K1 cells were stained with PE anti-human CD335 (NKp46) antibody (BioLegend #331908) and analyzed by flow cytometry. Parental CHO cells (green) were compared to low, medium and high NKp46 expressing CHO cells (yellow, red, blue).

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

Sequence

MSSTLPALLCVGLCLSQRISAQQQTLPKPFIWAEPHFMVPKEKQVTICCQGNYGAVEYQLHFEGSLFAVDRPKPPERINKVQFYIP
DMNSRMAGQYSCIYRVGELWSEPSNLLDLVVTEMYDTPTLSVHPGPEVISGEKVTFYCRLDTATSMFLLLKEGRSSHVQRGYGKV
QAEFPLGPVTTAHRGTYRCFGSYNNHAWSFPSEPVKLLVTGDIENTSLAPEDPTFPADTWGTYLLTTETGLQKDHALWDHTAQN
LLRMGLAFLVLVALVWFLVEDWLSRKRTRERASRASTWEGRRRLNTQTL

References

1. Sivori S., *et al.*, 1999 *Eur J Immunol.* 29 (5):1656-66.
2. Gardner G. and Fraker C.A., 2021 *Front Immunol.* 12:722979.
3. Sen Santara S., *et al.*, 2023 *Nature* 616 (7956):348-356.

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*
NKp46 Lentivirus	78717	500 µl x 2
NKp46 Lentivirus (Macaca fascicularis/Cynomolgus)	78779	500 µl x 2
NKp46 Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled Recombinant	100466	25 µg/50 µg
NCAM (CD56) CHO Cell Line (High, Medium or Low Expression)	78352	2 vials