

Description

CD123 (IL3Ra) CHO Cell Line is a CHO-K1 cell line over-expressing the human full length CD123 (accession number NM_002183.2), also known as IL3Ra (interleukin 3 receptor alpha). Surface expression of CD123 was confirmed by flow cytometry. This clonal cell line was selected for high level expression of CD123. Clones exhibiting lower levels of CD123 expression are also available (#79640-L and 79640-M). Each stable clonal cell line was selected for different levels of CD123 expression to mimic different stages of cancer target cells with various CD123 expression levels.

Background

CD123, also known as interleukin 3 receptor alpha (IL3Ra), is a transmembrane protein normally expressed on basophils and plasmacytoid dendritic cells and overexpressed on multiple blood cancers and leukemic hematopoietic stem cells. It normally plays a role in cell proliferation. CAR (chimeric antigen receptor)-T cells and other therapies targeting CD123 are currently being evaluated in clinical trials. CD123 may be especially valuable in treating B cell acute lymphoblastic leukemia patients who have relapsed after anti-CD19 CAR-T treatment.

Application

- Use as target cells to screen antibodies against CD123.
- Perform assays to characterize CD123-targeting CAR-T cells.
- Useful for binding assays to screen CD123 ligands.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains $\geq 1 \times 10^6$ 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 3B	BPS Bioscience #79529

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 3 (BPS Bioscience #60186):

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

Growth Medium 3B (BPS Bioscience #79529):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 µg/ml of Hygromycin.

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 150 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% CO₂ 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% 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 3B.

Cell Passage

1. Aspirate the medium, wash the cells with Phosphate Buffered Saline (PBS) without Ca²⁺/Mg²⁺, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3B and transfer to a tube.
3. Spin down cells at 150 x *g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3B.

- Seed into new culture vessels at the recommended sub-cultivation ratio of 1:30 twice per week.

Cell Freezing

- 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.
- Once the cells have detached, add Growth Medium 3B and count the cells.
- Spin down the cells at $150 \times g$ 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.
- 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.
- 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

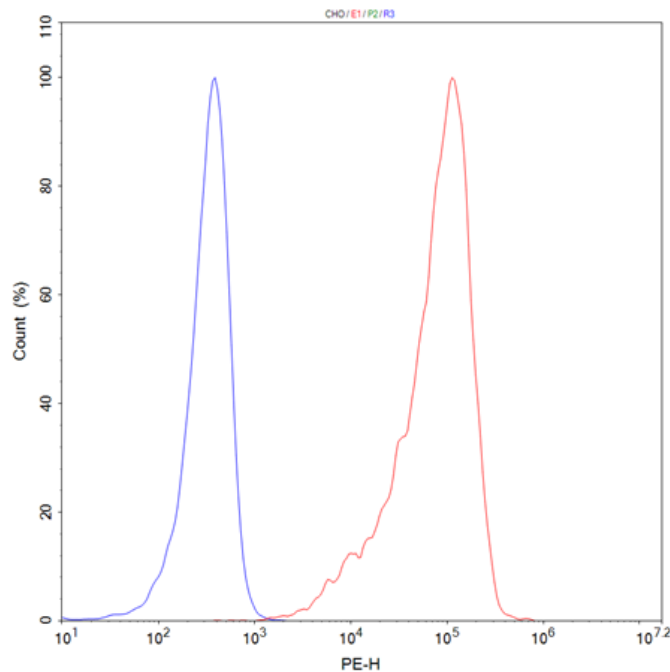


Figure 1: Cell surface expression of CD123 in CD123 (IL3Ra) CHO Cell Line by flow cytometry. CD123 (IL3Ra) CHO cells (red) and control parental CHO-K1 cells (blue) were stained with a PE-conjugated antibody and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

Data shown is representative.

Sequence

Human CD123 (NM_002183.2).

MVLLWLTLLLIAPCLLQTKEDPNPPITNLRMKAKAQQLTWDLNRNVTDIECVKDADYSMPAVNNSYCQFGAISLCEVTNYTVRV
 ANPPFSTWILFPENSGKPWAGAENLTCWIHDVDFLSCSWAVGPGAPADVQYDLYLNVANRRQQYECLHYKTDAQGTRIGCRF
 DDISRLSSGSQSSHILVRGRSAAFIPCTDKFVVFVFSQIEILTPPNMTAKCNKTHSFMHWKMRSHFNRFKRYELQIKRMQPVITEQ
 VRDRTSFQLLNPGTYTVQIRARERVYEFLSAWSTPQRFECDDQEEGANTRAWRTSLLIALGTLALVCFVICRRYLVMQRLFPRIPH
 MKDPIGDSFQNDKLVVWEAGKAGLEECLVTEVQVVQKT

References

Pizzitola I., *et al.*, 2014 *Leukemia*. 28(8): 1596-1605.
 Mardiros, A., *et al.*, 2015 *Curr Opin Hematol*. 22(6): 484-488.
 Ruella, M., *et al.*, 2016. *J Clin. Invest*. 126(10): 3814-3826.

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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>
Anti-CD123 Antibody, PE-Labeled	101954	25 tests/ 100 tests
Anti-CD123 Antibody, FITC-Labeled	101955	25 tests/ 100 tests
Anti-CD123 IgG Antibody	101036	50 µg/ 100 µg

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