

## Data Sheet

### CD123 (IL3Ra)-CHO Recombinant Cell Line (Medium Expression) Catalog # 79640-M

#### Description

Stable, recombinant clonal CHO cell line constitutively expressing full length human CD123 protein (Genbank #NM\_002183.2). Surface expression of CD123 was confirmed by flow cytometry. This clonal cell line was selected for medium level expression of CD123. Clones exhibiting higher and lower levels of CD123 expression are also available (#79640-H and #79640-L). 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-Ts 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 CD19 CAR-T treatment.

#### Application

Useful for screening and validating antibodies against CD123 and CD123 CAR-T for immunotherapy research and drug discovery. Also useful for CD123 binding assays to screen for CD123 ligands.

#### Host Cell

CHO K1 cell line, Chinese Hamster Ovary

#### Format

Each vial contains ~ 2 x 10<sup>6</sup> cells in 1 ml of FBS + 10% DMSO.

#### Storage

Store in liquid nitrogen immediately upon receipt.

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## Cell Culture

**Thaw Medium 3 (#60186):** Ham's F-12K medium (Hyclone#SH30526.01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

**Growth Medium 3B (#79529):** Thaw Medium 3 (#60186) plus 500 µg/ml Hygromycin (Thermo Fisher/Life Technologies, #10687010).

## Recommended Culture Condition

Thawing cells: Prepare a 15 ml conical tube with 10 ml of pre-warmed Thaw Medium 3 (**no hygromycin**). Quickly thaw cells in a 37°C water bath with constant and slow agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire content to Thaw Medium 3 (**no hygromycin**). Avoid pipetting up and down, and gently rock the conical tube.

Spin the cells down at 150 x g for 5 minutes. Discard the medium and re-suspend the cell pellet in fresh Thaw Medium 3 (**no hygromycin**). Transfer the entire content to a T75 flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO<sub>2</sub>. After 48-72 hours of incubation, change to fresh Thaw Medium 3 (**no hygromycin**), without disturbing the attached cells. Switch to Growth Medium 3B at the first passage.

*Subculture:* When cells reach 90% confluency, remove the medium and wash twice with PBS (without magnesium or calcium). Treat cells with 1 ml of 0.05% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 14 ml pre-warmed medium and gently pipette up and down to dissociate cell clumps. Dispense 0.5 ml of the cell suspension into a new T75 flask containing 9.5 ml pre-warmed media. Incubate cells in a humidified 37°C incubator with 5% CO<sub>2</sub>. Cells should be split twice per week at a 1:30 split ratio. Freeze cells in Thaw Medium 3 + 10% DMSO. Cells have been demonstrated to be stable for at least 20 passages; AMSBIO recommends preparing frozen stocks at an early passage.

## Mycoplasma Testing

This cell line has been screened using the Quick Test Mycoplasma Detection Kit (Biotool.com, #B39032) to confirm the absence of mycoplasma contamination.

## Application References

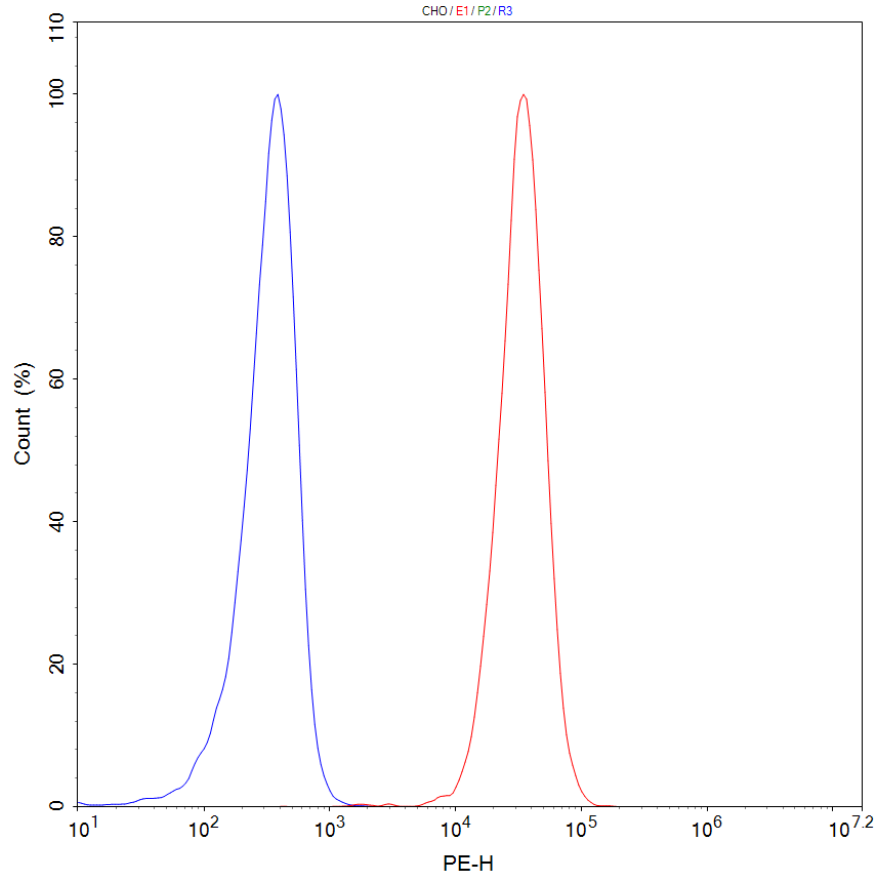
1. Pizzitola, I., *et al.* Chimeric antigen receptors against CD33/CD123 antigens efficiently target primary acute myeloid leukemia cells in vivo. *Leukemia*. 2014 Aug, **28(8)**: 1596-1605.
2. Mardiros, A., *et al.* T cells expressing CD123 chimeric antigen receptors for treatment of acute myeloid leukemia. *Curr Opin Hematol*. 2015 Nov, **22(6)**: 484-488.
3. Ruella, M., *et al.* Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. *J. Clin. Invest.* 2016 Oct, **126(10)**: 3814-3826.

**Vector and Sequence**

Human CD123 (NM\_002183.2) was cloned into pCMV3.

ATGGTCCTCCTTTGGCTCACGCTGCTCCTGATCGCCCTGCCCTGTCTCCTGCAAACGAAG  
 GAAGATCCAAACCCACCAATCACGAACCTAAGGATGAAAGCAAAGGCTCAGCAGTTGACCT  
 GGGACCTTAACAGAAATGTGACCGATATCGAGTGTGTTAAAGACGCCGACTATTCTATGCC  
 GGCAGTGAACAATAGCTATTGCCAGTTTGGAGCAATTTCCCTTATGTGAAGTGACCAACTAC  
 ACCGTCCGAGTGGCCAACCCACCATTCTCCACGTGGATCCTCTTCCCTGAGAACAGTGGG  
 AAGCCTTGGGCAGGTGCGGAGAATCTGACCTGCTGGATTTCATGACGTGGATTTCTTGAGC  
 TGCAGCTGGGCGGTAGGCCCGGGGGCCCCCGCGGACGTCCAGTACGACCTGTACTTGAA  
 CGTTGCCAACAGGCGTCAACAGTACGAGTGTCTTCACTACAAAACGGATGCTCAGGGAAC  
 ACGTATCGGGTGTCTGTTTCGATGACATCTCTCGACTCTCCAGCGGTTCTCAAAGTTCCCAC  
 ATCCTGGTGCAGGGGAGGAGCGCAGCCTTCGGTATCCCCTGCACAGATAAGTTTGTCTGTC  
 TTTTCACAGATTGAGATATTAACCTCCACCCAACATGACTGCAAAGTGTAAATAAGACACATTC  
 CTTTATGCACTGGAAAATGAGAAGTCATTTCAATCGCAAATTTTCGCTATGAGCTTCAGATAC  
 AAAAGAGAATGCAGCCTGTAATCACAGAACAGGTCAGAGACAGAACCTCCTTCCAGCTACT  
 CAATCCTGGAACGTACACAGTACAAATAAGAGCCCAGGAAAGAGTGTATGAATTCTTGAGC  
 GCCTGGAGCACCCCCAGCGCTTCGAGTGCAGACCAGGAGGAGGGCGCAAACACACAGTGC  
 CTGGCGGACGTGCTGCTGATCGCGCTGGGGACGCTGCTGGCCCTGGTCTGTGTCTTCG  
 TGATCTGCAGAAGGTATCTGGTGCAGAGACTCTTTCGCCGCATCCCTCACATGAAAGA  
 CCCCATCGGTGACAGCTTCCAAAACGACAAGCTGGTGGTCTGGGAGGCGGGCAAAGCCG  
 GCCTGGAGGAGTGTCTGGTGAAGTACAGGTCGTGCAGAAAACCTTAA  
 MVLLWLTLIIALPCLLQTKEDPNPPITNLRMKAKAQLTWDLNRNVTDIECVKDADYSMPAVN  
 NSYCQFGAISLCEVTNYTVRVANPPFSTWILFPENSGKPWAGAENLTCWIHDVDFLSCSWAVG  
 PGAPADVQYDLYLNVANRRQQYECLHYKTDAGQTRIGCRFDDISRLSSGSQSSHILVRGRSAA  
 FGIPCTDKFVFSQIEILTPPNMTAKCNKTHSFMHWKMRSHFNRKFRYELQIQKRMQPVITEQV  
 RDRTSFQLLNPGTYTVQIRARERVYEFLSAWSTPQRFECQEEGANTRAWRTSLIIALGTLIIAL  
 VCVFVICRRYLVMQRLFPRIIPMKDPIGDSFQNDKLVVWEAGKAGLEECLVTEVQVVQKT

**Quality Assurance**



**Figure 1. Expression of CD123 validated by flow cytometry.** Flow cytometry using PE-conjugated anti-human CD123 antibody (Biolegend, #306006) detects CD123 on the surface of CD123-CHO Recombinant Cell Line, #79640-M. (CD123-CHO, red; CHO parental, blue).

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### **Related Products**

<b>Product</b>	<b>Cat. #</b>	<b>Size</b>
CD123 (IL3Ra)-CHO Recombinant Cell Line (High Expression)	79640-H	2 vials
CD123 (IL3Ra)-CHO Recombinant Cell Line (Low Expression)	79640-L	2 vials
Growth Medium 3B	79529	500 ml
Thaw Medium 3	60186	100, 500ml
FOLH1 (PSMA)-CHO Recombinant Cell Line (High Expression)	79641-H	2 vials
FOLH1 (PSMA)-CHO Recombinant Cell Line (Medium Expression)	79641-M	2 vials
FOLH1 (PSMA)-CHO Recombinant Cell Line (Low Expression)	79641-L	2 vials
CD19-CHO Recombinant Cell Line (High Expression)	79561-H	2 vials
CD19-CHO Recombinant Cell Line (Medium Expression)	79561-M	2 vials
CD19-CHO Recombinant Cell Line (Low Expression)	79561-L	2 vials
CD22-CHO Recombinant Cell Line (High Expression)	79557-H	2 vials
CD22-CHO Recombinant Cell Line (Medium Expression)	79557-M	2 vials
BCMA-CHO Recombinant Cell Line (Medium Expression)	79500-M	2 vials
BCMA-CHO Recombinant Cell Line (High Expression)	79500-H	2 vials
BCMA-CHO Recombinant Cell Line (Low Expression)	79500-L	2 vials

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