

## **Data Sheet**

### **CD19 CHO Recombinant Cell Line (Medium Expression)**

### **Catalog #AMS.79561-M**

#### **Description**

Recombinant clonal stable CHO cell line constitutively expressing full length human CD19 protein, also known as B4 or CVID3, (Genbank #NM\_001770). Surface expression of CD19 was confirmed by flow cytometry. Each stable clonal cell line was selected for different levels of CD19 expression (High, Medium, Low) to mimic different stages of cancer target cells with various CD19 expression levels.

#### **Background**

B-lymphocyte antigen CD19 (Cluster of Differentiation 19), also known as B-Lymphocyte Surface Antigen B4 and CVID3, is a transmembrane protein expressed in follicular dendritic cells and all B lineage cells, except plasma cells. CD19 plays two major roles in human B cells. It acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane and it works within the CD19/CD21 complex to decrease the threshold for B cell receptor signaling pathways. Due to its presence on all B cells, it is a biomarker for B lymphocyte development and lymphoma diagnosis and can be utilized as a target for leukemia immunotherapies. CD19-targeted therapies based on T cells that express CD19-specific chimeric antigen receptors (CARs) have been utilized for their antitumor abilities in patients with CD19+ lymphoma and leukemia, such as Non-Hodgkins Lymphoma (NHL), CLL and ALL.

#### **Application**

1. Useful as CD19-expressing target cells in co-culture assay with CD19-CAR-T cell, for both CD19-specific cell killing assay and cytokine production assay.
2. Useful for screening and validating antibodies against CD19 and anti-CD19 CAR-T for immunotherapy research and drug discovery.
3. Useful for CD19 binding assays to screening for CD19 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 10% DMSO in FBS.

#### **Storage**

Store in liquid nitrogen immediately upon receipt.

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## Cell Culture

**Thaw Medium 3 (AMS, #AMS.60186):** F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

**Growth Medium 3D (AMS, #AMS.79539):** F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml G418

## Recommended Culture Condition

**Frozen Cells:** Prepare a 50 ml conical tube with 10 ml of pre-warmed Thaw Medium 3 (**no G418**). 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 G418**). 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 G418**). Transfer the entire content to a T25 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 G418**), without disturbing the attached cells. Continue to change the medium every 2-3 days until the cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture. Switch to Growth Medium 3D after the first passage.

**Subculture:** When cells reach 90% confluency, remove the medium and GENTLY wash once with PBS (without Magnesium or Calcium). These cells are loosely adherent and detach easily so do not re-suspend the PBS directly onto the cell surface. Treat cells with 2 ml of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml pre-warmed medium and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and re-suspend cells in 10 ml of pre-warmed Growth Medium 3D. Dispense 5 ml of the cell suspension into a new T75 flask containing 20 ml pre-warmed media. Incubate cells in a humidified 37°C incubator with 5% CO<sub>2</sub>. Freeze cells in freezing medium (10% DMSO in FBS) when cells reach 90% confluency. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks at an early passage so cells are not used beyond passage 20.

## Mycoplasma Testing

This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, #LT07-518) was used as a positive control.

## Application References

1. Tedder TF, *et al.* Isolation of cDNAs encoding the CD19 antigen of human and mouse B lymphocytes. A new member of the immunoglobulin superfamily. *J. Immunol.* 1989 Jul 15; **143**(2):712

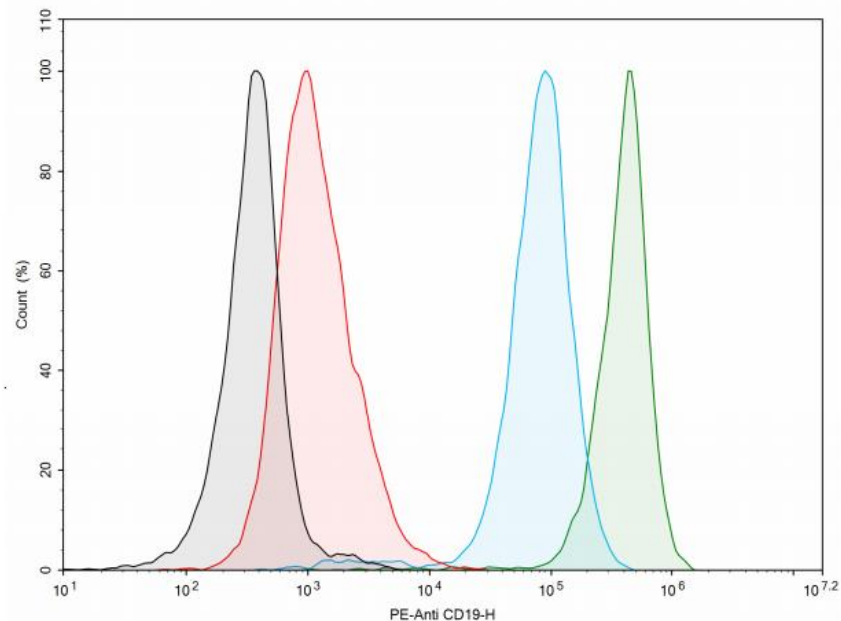
2. Kemeng W, *et al.* CD19: a biomarker for B cell development, lymphoma diagnosis and therapy.  
*Experimental Hematology & Oncology* 2012 **1**:36
3. Michel S. CD19 CAR T Cells.  
*Cell* 2017 Dec 14; **171**(7):1471

### Vector and Sequence

Human CD19 (NM\_001770) was cloned into pIRESneo3.

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTS DGPTQQLTWSRESPLKPFLK  
LSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWN  
VSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWE GEPCLPPRDSL NQSLSQD  
LTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMWVMETG LLLP  
RATAQDAGKYYCHRG NLTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHL  
QRALVLRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLPTPTSG LGRAQRWAAGLGGT  
APSYGNPSSDVQADGALGSRSPPGVGPEEEEEEGEGYEEP DSEEDSEFYENDSNLGQDQLSQD  
GSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVARTMDFLSPHGS AWDP SREATSLGS  
QSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGRMGTWSTR&

### Quality Assurance



**Figure 1. Expression of CD19 validated by flow cytometry.** Flow cytometry using PE-conjugated anti-human CD19 antibody (Biolegend, #302207) detects CD19 surface expression of CD19 CHO Recombinant Cell Lines with different expression levels: #AMS.79561-H, high expresser: green; #AMS.79561-M, medium expresser: blue; #AMS.79561-L, low expresser: red; WT CHO negative control: black.

## **Related Products**

<b>Product</b>	<b>Cat. #</b>	<b>Size</b>
CD19 CHO Recombinant Cell Line (High Expression)	AMS.79561-H	2 vials
CD19 CHO Recombinant Cell Line (Low Expression)	AMS.79561-L	2 vials
Human CD19, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled HiP™	AMS.79475	50 µg
Human CD19, Fc-Fusion, Avi-Tag HiP™	AMS.79472	50 µg
BCMA— CHO Recombinant Cell Line (Medium Expression)	AMS.79500-M	2 vials
BCMA— CHO Recombinant Cell Line (Low Expression)	AMS.79500-L	2 vials

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