Description

Clonal stable CHO cell line constitutively expressing full length human CD19 protein (also known as CVID3, Genbank accession #NM_001770) and human CD20 protein (also known as MS4A1 and FMC7, Genbank accession #NM_021950). This cell line was derived from our CHO-K1 Luciferase cells (BPS Bioscience, #79725), therefore it also constitutively expresses the firefly luciferase reporter. Surface expression of CD19 and CD20 were confirmed by flow cytometry.

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 used 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.

CD20 (MS4A1) is a glycosylated phosphoprotein expressed on the cell surface of B cells. Although the functional significance of CD20 is not clear, and CD20 has no known ligands, CD20 has been shown to regulate intracellular calcium levels. CD20 is a highly attractive target antigen for immunotherapy because it is expressed in more than 90% of patients with B-cell lymphoma. First approved in 1997, Rituximab (Rituxan) is a chimeric monoclonal antibody targeting CD20 and has been classified by the World Health Organization as an “Essential Medicine”. Since then, additional monoclonal antibodies against CD20 have been approved or are being tested in clinical trials for the treatment of multiple sclerosis (MS), chronic lymphocytic leukemia (CLL), follicular lymphoma, diffuse large B cell lymphoma (DLBCL), rheumatoid arthritis, non-Hodgkin’s lymphoma, systemic lupus erythematosus, and myalgic encephalomyelitis (chronic fatigue syndrome). Additionally, more recently, anti-CD20-CD19 bispecific CAR-T cells have been developed to address concerns over potential relapse.

Application

1. Useful for validation of anti-CD19 and anti-CD20 bispecific antibody.

2. Useful as CD19- and/or CD20-expressing target cells in co-culture assay with CD19- and/or CD20-CAR-T cells, for both CD19/CD20-specific cell killing assay and cytokine production assay.

3. Useful for screening and validating antibodies against CD19 or CD20 and anti-CD19 or anti-CD20 CAR-T for immunotherapy research and drug discovery.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~2 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

CHO K1 cell line, Chinese Hamster Ovary, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened using the MycoAlert™ Mycoplasma Detection kit (Lonza, #LT07-218) to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems 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.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 3	BPS Bioscience #60186
Growth Medium 3K	BPS Bioscience #78041

Materials Required for Cellular Assay

Name	Ordering Information
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated white clear-bottom assay plate	
Luminometer	

Storage Conditions

Cells will arrive upon 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. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 3K.

Media Required for Cell Culture

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

Growth Medium 3K (BPS Bioscience #78041):
F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1000 µg/ml Geneticin, 5 µg/ml Puromycin and 500 µg/ml of Hygromycin B to ensure cell expression.

Assay Medium: Thaw Medium 3 (BPS Bioscience #60186)

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 3 (**no Geneticin, Puromycin or Hygromycin B**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 3 (**no Geneticin, Puromycin or Hygromycin B**).
3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, add an additional ~3 ml of Thaw Medium 3 (**no Geneticin, Puromycin or Hygromycin B**) and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split.
5. Cells should be split before they are fully confluent. At first passage, switch to Growth Medium 3K (**contains Geneticin, Puromycin and Hygromycin B**).

Cell Passage

1. To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.25% Trypsin/EDTA.
2. After detachment, add Growth Medium 3K (**contains Geneticin, Puromycin and Hygromycin B**) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 3K and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: about 1:20 every 5 days.

Cell Freezing

1. To freeze down the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.25% Trypsin/EDTA.
2. After detachment, add Thaw Medium 3 (**no Geneticin, Puromycin or Hygromycin B**) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience, #79796) at ~2 x 10^6 cells/ml.
3. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for storage.

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

Validation Data

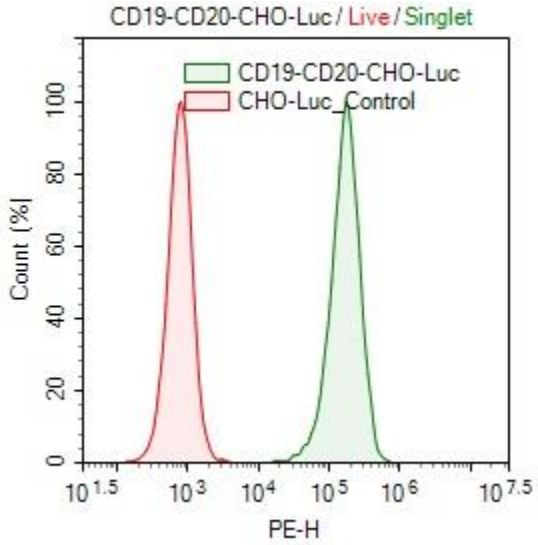

Figure 1. **Expression of CD19 validated by flow cytometry.**
Flow cytometry using PE-conjugated anti-human CD19 antibody (BioLegend, #302208) to detect CD19 surface expression on either the CD19 / CD20 / Firefly Luciferase - CHO Cell Line (green) or parental CHO-luc cells (red).

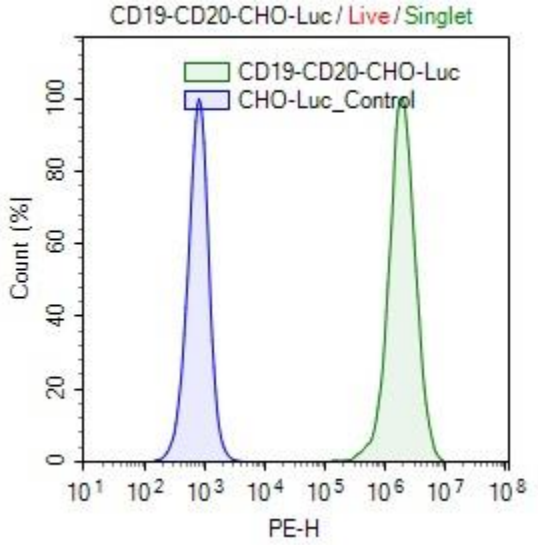

Figure 2. **Expression of CD20 validated by flow cytometry.**
Flow cytometry using PE-conjugated anti-human CD20 antibody (BioLegend, #302346) to detect CD20 surface expression on either the CD19 / CD20 / Firefly Luciferase - CHO Cell Line (green) or parental CHO-Luc cells (blue).

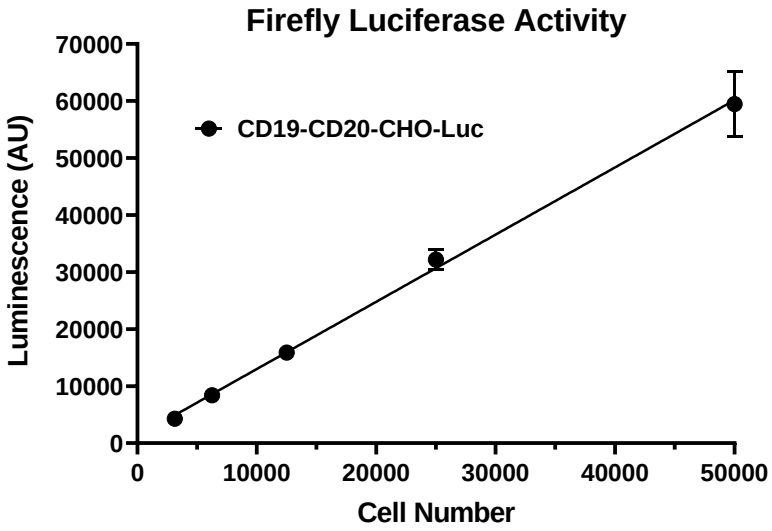

***Figure 3*. Luciferase activity of CD19 / CD20 / Firefly Luciferase - CHO Cells.**
CD19 / CD20 / Firefly Luciferase - CHO Cells were seeded in a 96-well plate at various densities. After four hours, luciferase activity under CMV promoter was measured using the ONE-Step luciferase assay system (BPS Bioscience, #60690).

Sequence

Human CD19 Sequence (Accession Number: NM_001178098)

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLKLSLGLPGLG
IHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWNVSDLGGLGCGLKNRS
SEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLNQSLSQDLTMAPGSTLWLSCGVPPDSVSRG
PLSWTHVHPKGPKSLLSLELKDDRPARDMWVMETGLLLPRATAQDAGKYYCHRGNLTMSFHLEITARPVL
WHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRRKRKRMTDPTRRFFKVTPPPGSGPQNQYGN
VLSLPTPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQADGALGSRSPPGVGPEEEEGEGYEEPDSEEDSEF
YENDSNLGQDQLSQDGSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVARTMDFLSPHGSAWDPSR
EATSLAGSQSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGRMGTWSTR

Human CD20 Sequence (Accession Number: NM_021950)

MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTLGAVQIMNGLFHIALGGLL
MIPAGIYAPICVTVWYPLWGGIMYIISGSLLAATEKNSRKCLVKGKMIMNSLSLFAAISGMILSIMDILNIKISH
FLKMESLNFIRAHTPYINIYNCEPANPSEKNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVENEWKRTCS
RPKSNIVLLSAEEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIIPIQEEEEEETETNFPEPPQDQESSPIENDSSP

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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*
CD19 CHO Recombinant Cell Line (Various Expression)	79561	2 vials
CD19/Luciferase-CHO Recombinant Cell line	79714	2 vials
CD38 / CD19 / Firefly Luciferase CHO Recombinant Cell Line	78149	2 vials
CD19 CHO Recombinant Cell Line (Low, Medium and High Expression)	79651-H 79561-M 79561-L	2 vials 2 vials 2 vials
Human CD19, Fc-Fusion, Avi-Tag HiP™	79472	50 µg
Human CD19, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled HiP™	79475	50 µg
Human CD19, Fc-Avi-Tag, PE-labeled	100732	50 µg
Human Monoclonal Anti-CD19 IgG Antibody	100981	50 µg
CD19, Avi-His-Tag	101015	50 µg
CD20 CHO Recombinant Cell Line (High or Medium Expression)	79624-M 79624-H	2 vials 2 vials
Anti-CD20 Agonist Antibody	71209	100 µg
CD20, Fc Fusion, Avi-Tag, PE-labeled	101027	50 µg