

Description

Recombinant clonal stable CHO-K1 cell line constitutively expressing both the full length human CD20 protein, also known as MS4A1 (Genbank #NM_152866), and the firefly (*Photinus pyralis*) luciferase. Surface expression of CD20 was confirmed by flow cytometry.

Background

CD20 (MS4A1) is a glycosylated phosphoprotein expressed on the cell surface of B cells. CD20 is a highly attractive target antigen for immunotherapy because it is highly 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). More recently, anti-CD20-CD19 bispecific CAR-T cells have been developed to address concerns over potential relapse.

Application

- Useful for CD20 binding assays to screen for potential CD20 ligands.
- Useful as CD20-expressing target cells in co-culture assays with Anti-CD20-CAR-T cells

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >2 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 3A	BPS Bioscience #60188

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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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 for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37 °C with 5% CO₂. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 3 (BPS Bioscience #60186):

F-12K Medium (Kaighn's Modification of Ham's F-12 Medium) supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 3A (BPS Bioscience #60188):

F-12K Medium (Kaighn's Modification of Ham's F-12 Medium) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin and 500 µg/ml of Hygromycin.

Cell Culture Protocol

Cell Thawing

- 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 (**no Geneticin or Hygromycin**).
Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.
- 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 (**no Geneticin or Hygromycin**).
- Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO₂ incubator.
- After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 (**no Geneticin or Hygromycin**), and continue growing in a 5% CO₂ incubator at 37°C until the cells are ready to passage.
- Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 3A (**contains Geneticin and Hygromycin**).

Cell Passage

- Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
- Once the cells have detached, add Growth Medium 3A and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3A (**contains Geneticin and Hygromycin**). Seed into new culture vessels at the desired sub-cultivation ratio of 1:10 or 1:20 twice a week.

Cell Freezing

- Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
- Once the cells have detached, add Growth Medium 3A and count the cells.

3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at $\sim 2 \times 10^6$ cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
5. 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.

Validation Data

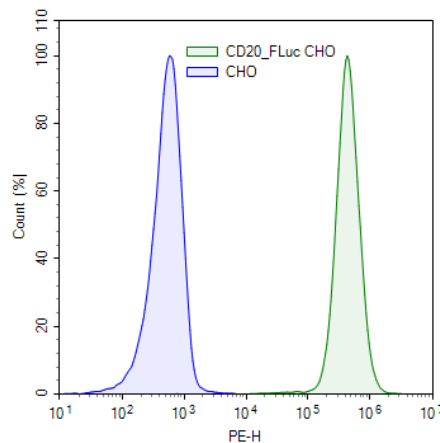


Figure 1. Flow cytometry analysis of CD20 cell surface expression in CD20/Firefly Luciferase CHO Cell Line. CD20/Firefly Luciferase CHO cells or control CHO cells were stained using PE-conjugated anti-human CD20 antibody (Biolegend, #302305) and analyzed by flow cytometry. Parental CHO cells (blue) and CD20/Firefly Luciferase CHO Cell Line (green).

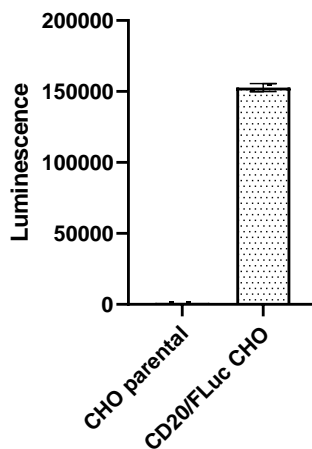


Figure 2. Luciferase activity of CD20/Firefly luciferase CHO cells. CD20/Firefly Luciferase cells were seeded into a 96-well plate at 500 cells/well in 50 μ L of thaw medium 3, and the luciferase activity was measured using the ONE-Step luciferase assay system (BPS Bioscience, #60690).

Sequence

Human CD20 sequence (NM_152866)

MTTPRNSVNGTFPAEPMKGPAMQSGPKPLFRMSSLVGPQTQFFMRESKTLGAVQIMNGLFHIALGGLLMIPAGIYAPICVTV
WYPLWGGIMYIISGSLAATEKNSRKCLVKGKMIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTPYINIYNCEPANPS
EKNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVENEWKRTCSRPKSNIVLLSAEEKKEQTIEIKKEEVVGLTETSSQPKNEEDIEII
PIQEEEEETETNFPEPPQ DQESSPIENDSSP

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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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Firefly Luciferase - K562 Recombinant Cell Line	78621	2 vials
Firefly Luciferase - Raji Recombinant Cell Line	78622	2 vials
Firefly Luciferase - RPMI 8226 Recombinant Cell Line	79834	2 vials
Firefly Luciferase - CHO Recombinant Cell Line	79725	2 vials
CD20 CHO Recombinant Cell Line	79624	2 vials
CD19/ Firefly Luciferase - CHO Recombinant Cell Line	79714	2 vials
CD22/ Firefly Luciferase - CHO Recombinant Cell Line	79715	2 vials
CD19/CD20/ Firefly Luciferase - CHO Recombinant Cell Line	78186	2 vials
BCMA/CD20/Firefly Luciferase-CHO Recombinant Cell Line	78185	2 vials
Anti-CD19 CAR Lentivirus	78600	50 μ l
Anti-CD20 CAR Lentivirus	78606	50 μ l
Anti-CD22 CAR Lentivirus	78608	50 μ l
Anti-BCMA CAR Lentivirus	78603	50 μ l