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Data Sheet

CD20 - CHO Recombinant Cell Line (High Expression)

Cat #: 79624-H

Product Description

Recombinant clonal stable CHO cell line constitutively expressing full length human CD20 protein, also known as MS4A1 (Genbank #NM_152866). Surface expression of CD20 was confirmed by flow cytometry. Each stable clonal cell line was selected for different levels of CD20 expression (High, Medium) to mimic different stages of cancer target cells with various CD20 expression levels.

Background

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 on 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 as CD20-expressing target cells in co-culture assays with CD20-CAR-T cells for CD20-specific cell killing assays.
2. Useful for screening and validating antibodies against CD20 and anti-CD20 CAR-T for immunotherapy research and drug discovery.
3. Useful for CD20 binding assays to screen for potential CD20 ligands.

Host Cell

CHO K1 cell line, Chinese Hamster Ovary

Format

Each vial contains ~2 X 10^6 cells in 1 ml of 10% DMSO in FBS.

Storage

Store in liquid nitrogen immediately upon receipt.

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Cell Culture Conditions

Thaw Medium 3 (BPS Bioscience, #60186): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin
Growth Medium 3D (BPS Bioscience #79539): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of G418

Recommended Culture Conditions

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. 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_2. 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 0.05% 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 spin down cells. 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_2. Freeze cells in freezing medium (10% DMSO in FBS) in cryogenic vials when cells reach 90% confluency. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage. 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.

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Application References

1. Kosmas, C, *et al.* Anti-CD20-based therapy of B cell lymphoma: state of the art. *Leukemia. 2002*; **16**: 2004-2015
2. Cragg, MS, *et al*. The biology of CD20 and its potential as a target for mAb therapy. *Curr Dir Autoimmun*. *2005*; **8**: 140-174
3. Martyniszyn, A, *et al.* CD20-CD19 bispecific CAR T cells for the treatment of B-cell malignancies. *Hum Gene Ther*. *2017* Dec; **28(12)**: 1147-1157
4. Lee, SY, *et al.* Preclinical optimization of a CD20-specific chimeric antigen receptor vector and culture conditions. *J Immunother. 2018* Jan; **41(1)**: 19-31

Vector and Sequence

Human CD20 (NM_152866) was cloned into pIRESneo3.

MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTLGAVQIMNGLFHIALGGLLM
IPAGIYAPICVTVWYPLWGGIMYIISGSLLAATEKNSRKCLVKGKMIMNSLSLFAAISGMILSIMDILNIK
ISHFLKMESLNFIRAHTPYINIYNCEPANPSEKNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVE
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DQESSPIENDSSP

Quality Assurance

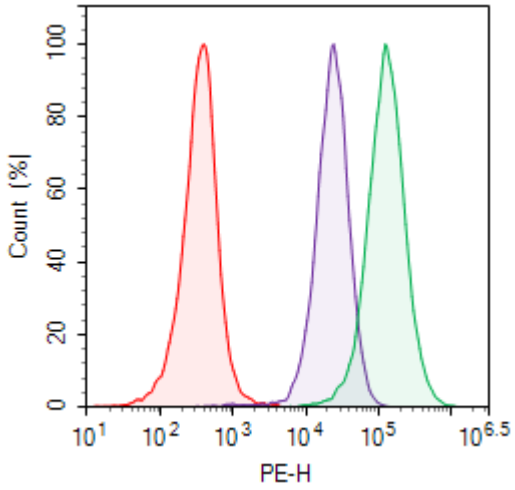

Figure 1. Expression of CD20 validated by flow cytometry. Flow cytometry using PE-conjugated anti-human CD20 antibody (Biolegend, #302305) detects CD20 surface expression of CD20-CHO Recombinant Cell Lines with different expression levels: 79624-H, high expresser: green; 79624-M, medium expresser: purple; WT CHO negative control: red.

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Related Products

Product	Cat. #	Size
CD20 - CHO Recombinant Cell Line (Medium Expression)	79624-M	2 vials
Anti-CD20 Agonist Antibody	71209	100 µg
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
CD19, Fc-Fusion (IgG1), Avi-Tag	79472	100 µg
CD19, Fc-Fusion (IgG1), Avi-Tag, Biotin-Labeled	79475	50 µg