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Datasheet

CD19 / BCMA / Firefly Luciferase - CHO Recombinant Cell Line Catalog #: 78030

Product Description

Recombinant clonal stable CHO cell line constitutively expressing full length human CD19 protein (also known as B4 or CVID3, Genbank #NM_001770), human BCMA protein (B-Cell Maturation Antigen or CD269, GenBank accession #NM_001192) and the firefly luciferase. Surface expression of CD19 and BCMA 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.

B-Cell Maturation Antigen (BCMA), also known as CD269, is a cell surface receptor of the TNF receptor superfamily that recognizes B-Cell Activating Factor (BAFF). BCMA is preferentially expressed on mature B-lymphocytes and Multiple Myeloma (MM) cells. BCMA is a highly attractive target antigen for immunotherapy, not only because of its restricted expression in nonmalignant tissue, but also due to its almost universal expression on MM cells. Pre-clinical studies using CAR (Chimeric Antigen Receptor) T-cells targeting BCMA have demonstrated anti-MM activity, and in 2017, the FDA granted BCMA CAR T-Cell immunotherapy the breakthrough designation in treating Multiple Myeloma.

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Applications

1. Useful for validation of anti-CD19 and anti-BCMA bispecific antibody.
2. Useful as CD19- and/or BCMA-expressing target cells in co-culture assay with CD19- and/or BCMA-CAR-T cells, for both CD19/BCMA-specific cell killing assay and cytokine production assay.
3. Useful for screening and validating antibodies against CD19 (or BCMA) and anti-CD19 (or anti-BCMA) CAR-T for immunotherapy research and drug discovery.

Host Cell

CHO K1 cell line, Chinese Hamster Ovary

Format

Each vial contains ~2 x 10⁶ cells in 1 ml of 10% DMSO and 90% FBS

Storage

Immediately upon receipt, store in liquid nitrogen.

Cell Culture

Thaw Medium 3 (BPS Bioscience #60186): Ham's F-12 medium (Hyclone, #SH30526.01) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 3K (BPS Bioscience, #78041): Thaw medium 3 (BPS Bioscience, #60186) plus 1000 µg/ml Geneticin (Thermo Fisher, #11811031), 5 µg/ml Puromycin (InvivoGen, #ant-pr-1) and 500 µg/ml of Hygromycin B (Thermo Fisher, #10687010) to ensure recombinant expression.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water bath, transfer to a tube containing 10 ml of Thaw Medium 3 (**no Geneticin, Puromycin or Hygromycin B**), spin the cells down, remove the supernatant, and then resuspend the cells in 5ml pre-warmed Thaw Medium 3 (**no Geneticin, Puromycin or Hygromycin B**). Then transfer the resuspended cells to a T25 flask and culture in a 37°C CO₂ incubator overnight. The next day, replace the medium with fresh Thaw Medium 3 (**no Geneticin, Puromycin or Hygromycin B**) and continue growing in a CO₂ incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At the first passage, switch to Growth Medium 3K (**contains Geneticin, Puromycin and Hygromycin B**).

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To passage the cells, rinse the cells with Phosphate Buffered Saline (PBS), detach the cells from the culture vessel with 0.25% Trypsin/EDTA, add Growth Medium 3K and transfer to a tube. Next, spin the cells down, remove the supernatant, and then resuspend the cells and seed appropriate aliquots of the cell suspension into new culture vessels. Suggested subcultivation ratios: 1:10 to 1:20 twice a week.

To freeze the cells down, rinse the cells with Phosphate Buffered Saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA. After detachment, add Thaw Medium 3 (no Geneticin, Hygromycin B or Puromycin) and count the cells, then transfer to a tube, spin the cells down, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at $\sim 2 \times 10^6$ cells/ml. Dispense 1 ml of cell aliquots into each cryogenic vial. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage. It is recommended to expand the cells and freeze down at least 10 vials of cells for future use at early passages.

Mycoplasma Testing

This cell line has been screened using Plasmotest™ - Mycoplasma Detection Kit (InvivoGen #rep-pt1) to confirm the absence of *Mycoplasma* species.

Materials Required by Not Supplied

- Thaw Medium 3 (BPS Bioscience, #60186)
- Growth Medium 3K (BPS Bioscience, #78041)
- 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step luciferase assay system (BPS Bioscience, #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

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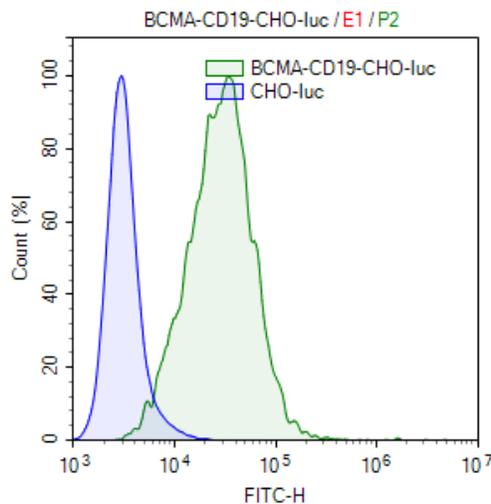
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Figure 1. Expression of BCMA validated by flow cytometry.

Flow cytometry using FITC-conjugated anti-human BCMA antibody (R&D Systems, #FAB1931G) to detect BCMA surface expression on either the CD19 / BCMA / Firefly Luciferase - CHO Recombinant Cell Line (green) or parental CHO-luc cells (blue).



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Figure 2. Expression of CD19 validated by flow cytometry.

Flow cytometry using PE-conjugated anti-human CD19 antibody (Biolegend, #302207) to detect CD19 surface expression on either the CD19 / BCMA / Firefly Luciferase - CHO Recombinant Cell Line (green) or parental CHO-luc cells (blue).

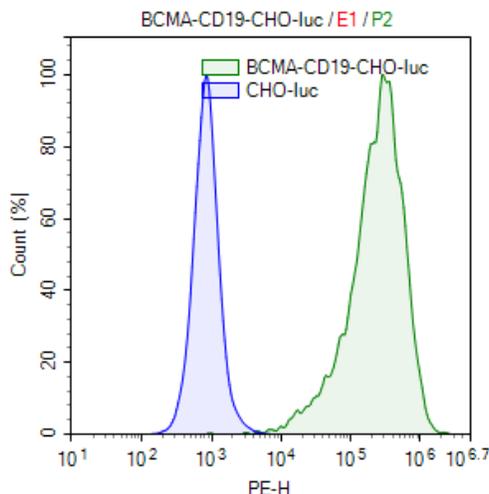
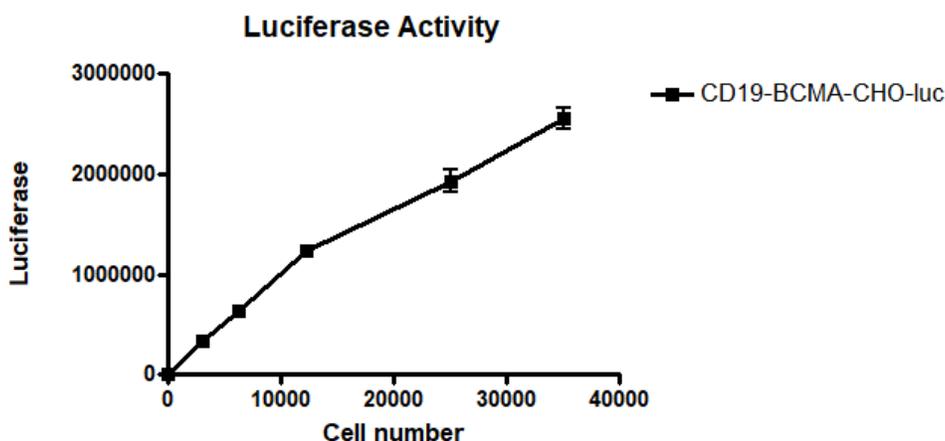


Figure 3. Luciferase activity of CD19 / BCMA / Firefly Luciferase - CHO Recombinant Cells.

CD19 / BCMA / Firefly Luciferase - CHO Recombinant Cells were seeded in a 96-well plate at various densities. After four hours, luciferase activity was measured using the ONE-Step luciferase assay system (BPS Bioscience, #60690).



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Application References

1. Kang, L., *et al.* 2020. Characterization of novel dual tandem CD19/BCMA chimeric antigen receptor T cells to potentially treat multiple myeloma. *Biomark Res.* **(8):14**
2. D'Agostino, M., *et al.* 2020. Anti-BCMA CAR T-cell therapy in multiple myeloma: can we do better? *Leukemia* **34:21-34.**
3. Sadelain, M. 2017. CD19 CAR T Cells. *Cell* **171(7):1471**

Sequence

CD19 (Genbank #NM_001770)

MPPPRLLFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPL
KPFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNV
EGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGER
PCLPPRDSLNSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSL
ELKDDRPARDMWVMEGLLLPRATAQDAGKYYCHRGNTMSFHLEITARPVLWHWLL
RTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRKRKRMTDPTRRFFKVTPPP
PQNQYGNVLSLPTPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQADGALGSRSP
GPEEEEEGEGYEEDSEEDSEFYENDSNLQDQLSQDGSYENPEDEPLGPEDEDSF
SNAESYENEDEELTQPVRTMDFLSPHGSADPSREATSLGSQSYEDMRGILYAAPQ
LRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGGRMGTWSTR

BCMA (GenBank accession #NM_001192)

MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNAILW
TCLGLSLIISLAVFVLMFLLRKINSEPLKDEFKNTGSGLLGMANIDLEKSRTGDEIILPRGL
EYTVVEECTCEDCIKSKPKVDSDFHCFPLPAMEEGATILVTTKTNDYCKSLPAALSATEIEK
SISAR

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Related Products

Product	Cat. #	Size
BCMA-CHO Recombinant Cell Line (High Expression)	79500-H	2 vials
BCMA-CHO Recombinant Cell Line (Low Expression)	79500-L	2 vials
BCMA/Luciferase-CHO Recombinant Cell line	79724	2 vials
Human BCMA (CD269)	90105-A	5 µg
Human BCMA (CD269)	90105-B	20 µg
Human BCMA, Fc-Fusion, Avi-Tag HiP™	79465	100 µg
Human BCMA, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled HiP™	79467	50 µg
Anti-BCMA Antibody	100173-1	50 µg
Anti-BCMA Antibody	100173-2	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/Luciferase-CHO Recombinant Cell line	79714	2 vials
Human CD19, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled HiP™	79475	50 µg
Human CD19, Fc-Fusion, Avi-Tag HiP™	79472	50 µg
Thaw Medium 3	60186	100, 500ml
Growth Medium 3K	78041	500 ml
ONE-Step™ Luciferase Assay System	60690-1	10 ml

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