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Data Sheet

CD19 / Firefly Luciferase - CHO Recombinant Cell Line

Catalog #79714

Description

Recombinant clonal stable CHO cell line constitutively expressing full length human CD19 protein (also known as B4 or CVID3, Genbank #NM_001770) and the firefly luciferase. Surface expression of CD19 was 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.

Application

1. Useful as CD19-expressing target cells in co-culture assay with CD19-CAR-T cells, 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 screen for CD19 ligands.

Host Cell

CHO K1 cell line, Chinese Hamster Ovary

Format

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

Storage

Store in liquid nitrogen immediately upon receipt.

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Materials Required but Not Supplied

- Thaw Medium 3 (BPS Bioscience, #60184)
- Growth Medium 3A (BPS Bioscience, #60188)
- 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step luciferase assay system (BPS Bioscience, #60690) or other luciferase reagent for measuring firefly luciferase activity
- Luminometer

Cell Culture

Thaw Medium 3 (BPS Bioscience, #60186): Ham's F-12 medium (Hyclone #SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 3A (BPS Bioscience, #60188): Thaw Medium 3 (BPS Bioscience, #60186) plus 1 mg/ml G418 (Thermo Fisher, #11811031) and 500 µg/ml of Hygromycin B (Thermo Fisher, #10687010) to ensure recombinant expression.

Cells should be grown at 37°C with 5% CO₂ using Growth Medium 3A. CD19/Luciferase CHO cells should exhibit a typical cell division time of ~24 hours.

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 or Hygromycin B)**, spin the cells down, remove the supernatant, and then re-suspend the cells in pre-warmed Thaw Medium 3 (**no Geneticin or Hygromycin B**). Then transfer the re-suspended 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 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. After the first passage, switch to **Growth Medium 3A (contains Geneticin and Hygromycin B)**.

To passage the cells, rinse the cells with Phosphate Buffered Saline (PBS), detach the cells from the culture vessel with 0.25% Trypsin/EDTA, and add Growth Medium 3A and transfer to a tube. Next, spin the cells down, remove the supernatant, and then re-suspend 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 or Hygromycin B)** 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 ~2 x 10⁶ 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 more than 10 vials of cells for future use at early passages.

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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 pIRESneo.

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLLK
LSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWN
VSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWE GEPCLPPRDSL NQSL SQD
LTMAPGSTLWLSGCVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMWV METGLLLP
RATAQDAGKYYCHRG NLTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHL
QRALVLRKRKRMTDPTRRFFKVT PPPGSGPQNQYGNVLSLPTPT SGLGRAQRWAAGLGGT
APSYGNPSSDVQADGALGSRSPPGVGP EEEEEGEGYEEPDSEEDSEFYENDSNLGQDQLSQD
GSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQP VARTMDFLSPHGS AWDP SREATSLGS
QSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGRMGTWSTR

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Quality Assurance

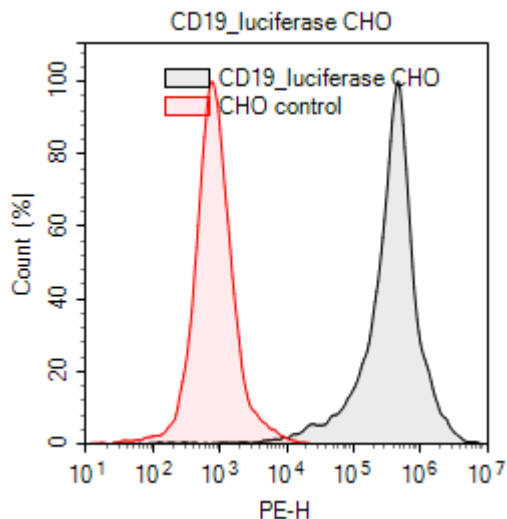


Figure 1. Expression of CD19 validated by flow cytometry. Flow cytometry using PE-conjugated anti-human CD19 antibody (Biolegend, #302207) to detect CD19 surface expression on either CD19/Luciferase CHO cells (black) or parental CHO-K1 cells (red).

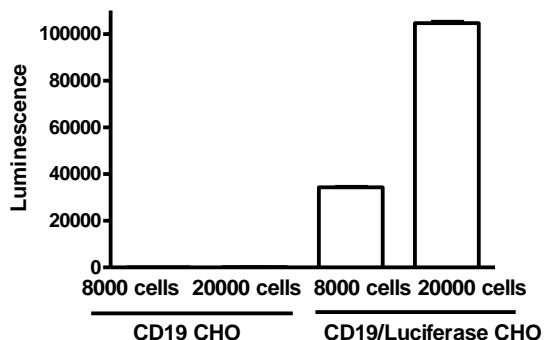


Figure 2. Luciferase activity of CD19/Luciferase CHO recombinant cells.

CD19/Luciferase CHO recombinant cells were seeded in a 96-well plate at 8000 cells/well or 20000 cells/well. The next day, luciferase activity was measured using the ONE-Step luciferase assay system (BPS Bioscience, #60690).

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Related Products

Product	Cat. #	Size
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
Human CD19, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled HiP™	79475	50 µg
Human CD19, Fc-Fusion, Avi-Tag HiP™	79472	50 µg
BCMA CHO Recombinant Cell Line (High Expression)	79500-H	2 vials
BCMA CHO Recombinant Cell Line (Medium Expression)	79500-M	2 vials
BCMA CHO Recombinant Cell Line (Low Expression)	79500-L	2 vials
BCMA/Luciferase-CHO Recombinant Cell line	79724	2 vials
CD22/Luciferase-CHO Recombinant Cell Line	79715	2 vials
Thaw Medium 3	60186	100, 500ml
Growth Medium 3A	60188	500 ml

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