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Data Sheet

ICOS/NFAT Reporter-Jurkat Recombinant Cell Line
Catalog #: 79668

Product Description
Recombinant Jurkat T cell expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of a chimeric receptor consisting human ICOS (also known as inducible T-cell costimulator or CD278, Genbank Accession #NM_012092) and the cytoplasmic domain of human CD3 zeta.

Background
ICOS is a costimulatory molecule of the CD28 cell surface receptor superfamily that is expressed on activated T-cells. ICOS is involved in T-cell responses upon binding with its ligand, ICOSL (also known as B7-H2, CD275), which is normally expressed on B-cells, dendritic cells and monocytes. ICOS expression confers an activated phenotype and a strong suppressive capacity to intra-tumoral regulatory T-cells. The ICOS/ICOSL pathway is a key target for cancer immunotherapy.

Application
- Screen for agonists of ICOS signaling in a physiologically relevant cellular context
- Characterize T cell-mediated immune responses of ICOS and its interaction with ICOSL

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.

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination.

Thaw Medium 2 (BPS Bioscience #60184): RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 2F (BPS Bioscience #79669): Thaw Medium 2 (BPS Bioscience #60184), 1 mg/ml of Geneticin (Life Technologies #11811031), and 0.5 µg/ml of Puromycin (Takara, #631306).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2F.

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Recommended Culture Condition

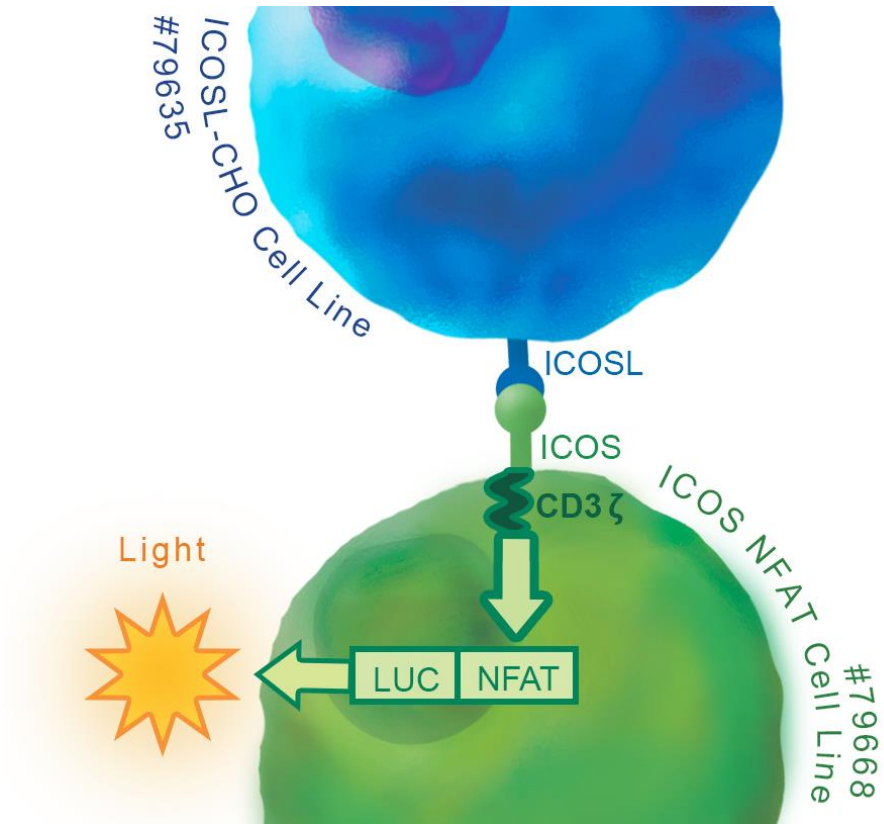
It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 **(**no Geneticin and Puromycin). Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2 (no Geneticin and Puromycin). Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional 3 – 4 ml of growth medium without antibiotics. At first passage, switch to growth medium 2F (contains Geneticin and Puromycin). Cells should be split before they reach 2 x 10^6 cells/ml. Note: This cell line tends to grow more slowly than the parental Jurkat cells.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml. Subcultivation ratio: 1:10 to 1:20 twice a week.

Functional Validation and Assay Performance

Expression of human ICOS in the Jurkat cell line was confirmed by Flow Cytometry. The functionality of the cell line was validated using an anti-ICOS agonist antibody and a ICOS:ICOSL cell-based assay.

Assay Principle



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Materials Required but Not Supplied

- Assay Medium: Thaw Medium 2 (BPS Bioscience #60184)
- ICOSL-CHO K1 Recombinant Cell Line (BPS Bioscience #79635) and its recommended growth medium (Growth Medium 3D, BPS Bioscience #79539)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience #60690) for measuring firefly luciferase activity
- Luminometer

Assay Protocol and Functional Analysis

A) ICOS/NFAT reporter activities stimulated by ICOSL-CHO cells

1. Harvest ICOSL-CHO cells from culture in growth medium and seed cells into white clear-bottom 96-well microplate in 100 µl growth medium. Incubate the plate at 37°C in a CO_2 incubator overnight. Leave a few wells empty as cell-free controls.

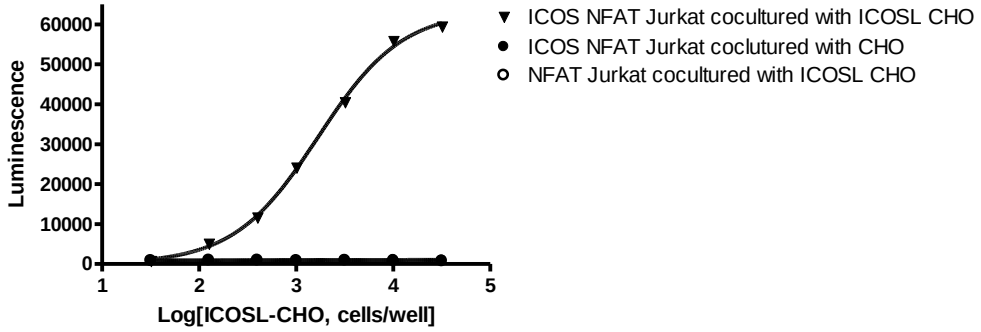
2. Next day, harvest the ICOS/NFAT Reporter-Jurkat cells by centrifugation and resuspend in assay medium. Dilute the cells to 3 x10^5 / ml in assay medium. Remove the medium from ICOSL-CHO cells and add 50 µl of ICOS/NFAT Reporter-Jurkat cells to the wells.

3. Incubate the plate at 37°C in a CO_2 incubator for 5 hours.

4. Perform luciferase assay using the ONE-Step luciferase assay system: Prepare the ONE-Step Luciferase reagent per recommended protocol. Add 50 µl of ONE-Step Luciferase reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer.

5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Figure 1. The ICOS/NFAT Reporter Activities Stimulated by ICOSL-CHO Cells.



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B) Functional assay of anti-ICOS agonist antibody on ICOS/NFAT Reporter-Jurkat cells

1. Harvest ICOS/NFAT Reporter-Jurkat cells from culture in growth medium and seed 20,000 cells per well into white clear-bottom 96-well plate in 45 µl of assay medium.

2. Dilute anti-ICOS agonist antibody in assay medium and treat cells with 5 µl of 10X dilutions of the anti-ICOS antibody.

3. Incubate the plate at 37°C in a CO_2 incubator for 5 hours.

4. Perform luciferase assay using the ONE-Step luciferase assay system: Prepare the ONE-Step Luciferase reagent per recommended protocol. Add 50 µl of ONE-Step Luciferase reagent per well (BPS Bioscience #60690) and rock at room temperature for 20 minutes. Measure luminescence using a luminometer

   Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NFAT luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 2 Dose response of anti-ICOS agonist antibody on ICOS/NFAT Reporter-Jurkat cells

Serial dilutions of anti-ICOS agonist antibody (Biolegend #313512) were added to ICOS/NFAT Reporter-Jurkat cells (BPS Bioscience #79668), and then incubated at 37°C for 5 hours. After the treatment, the Luciferase assay was performed.

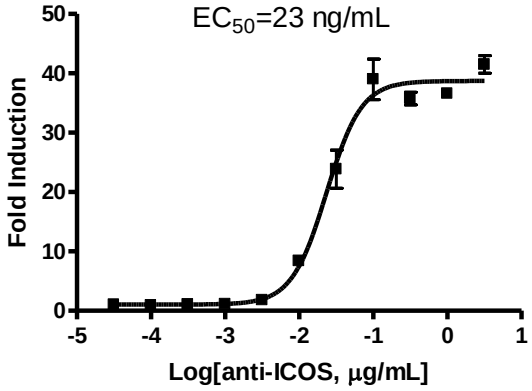

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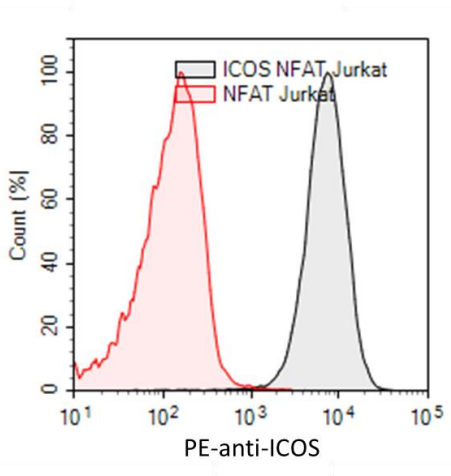
Figure 3. FACS Analysis of Cell Surface Expression of ICOS in ICOS/NFAT Reporter-Jurkat cells.

ICOS/NFAT Reporter-Jurkat cells or NFAT Reporter-Jurkat cells (BPS Bioscience #60621) were stained with PE-labeled anti-ICOS antibody (BD Bioscience #557802, clone#DX29) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.



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Related Products	**Cat. #**	**Size**
ICOSL (B7-H2) -CHO Recombinant Cell Line	79635	2 vials
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	60621	2 vials
ICOS (CD278), Fc fusion (Human)	71179	100 µg
B7-H2 (CD275, ICOSL), Fc fusion (Human) HiP™	71130	100 µg
B7-H2, Avi-His-Tag	79119	100 µg
B7-H2, Avi-His-Tag, Biotin-Labeled	79300	50 µg
CD276 (B7-H3), Avi-His-Tag HiP™	79337	100 µg
B7-H4, His-tag (Human)	71144	100 µg
Thaw Medium 2	60184	100 ml
Growth Medium 2F	79669	500 ml
Growth Medium 3D	79539	500 ml