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Data Sheet

IL-2-Luciferase Reporter (Luc) - Jurkat Cell Line
Catalog # 60481

Description
Human IL-2 reporter construct is stably integrated into the genome of Jurkat T-cells. The firefly luciferase gene is controlled by a human IL-2 promoter.

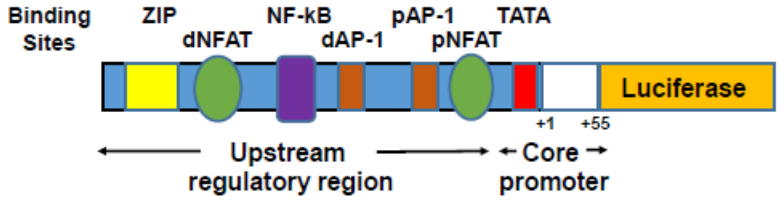

Figure 1. Illustration of IL-2 promoter region with representative transcription factor binding site.

Background
Interleukin-2 (IL-2) is a key cytokine important for proliferation and differentiation of T cells into effector T cells through interaction with the IL-2 receptor.

Host Cell
Human Acute T-Cell Leukemic Cell Line (Clone E61). Suspension cells.

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.

Culture Medium
Thaw Medium 2 (BPS Cat. #60184): RPMI 1640 medium (Thermo Fisher, Cat. #A1049101) supplemented with 10% FBS (Thermo Fisher, Cat. #26140079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 2B (BPS Cat. #79530): Thaw Medium 2 (BPS Cat. #60184) supplemented with 1 mg/ml G418 (Thermo Fisher, Cat. No.11811031).

Recommended Culture conditions
Frozen Cells: Prepare a 50 ml conical tube and a T-25 culture flask with 5 ml of pre-warmed Thaw Medium 2. 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 contents to the

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conical tube with Thaw Medium 2 **(no G418)** and rock the tube gently. Centrifuge the cells at 200 x g for 3 minutes. Re-suspend the cells in 5 ml of pre-warmed Thaw Medium 2 **(no G418)** and transfer the entire content to the T25 culture flask containing Thaw Medium 2 **(no G418).** Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. Forty-eight hours after incubation, centrifuge cells at 250 x g for 5 minutes and re-suspend to fresh Thaw Medium 2 **(no G418).** Continue to monitor growth for 2-3 days and change medium to remove dead debris. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture. Switch to Growth Medium 2B **(containing G418)** after multiple cell colonies (in clumps) start to appear (indicative of healthy cell division). We recommend passing cells for 3 passages after thawing before using them in the luciferase assay.

Subculture: When cells reach ~2.5 x 10^6 cells/ml, transfer cells to a 50 ml conical tube and centrifuge cells at 200 x g for 5 minutes. Wash cells once with PBS (without Magnesium or Calcium) and re-suspend cells in 10 ml pre-warmed Growth Medium 2B; gently pipette up and down to dissociate cell clumps. Dispense cell suspension at a 1:5 to 1:10 ratio into a new T-75 flask containing pre-warmed 15-20 ml Growth Medium 2B. Incubate cells in a humidified 37°C incubator with 5% CO_2. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks so cells are not used beyond passage 20.

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Cat. No. LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Cat. No. LT07-518) was used as a positive control.

Application
The IL-2-luciferase Jurkat reporter cell line is suitable for monitoring the transcription activity of IL-2 in response to stimulants, and establishing cell-based screens for inhibitors that target specific IL-2 stimulating molecules. This reporter cell line has been tested and validated by BPS using stimuli including a combination of phorbol 12-myristate 13-acetate (PMA) with ionomycin (**Figure 2**), anti-human CD3 antibody (**Figure 3**) and TCR activator / CHO-K1 cell line (**Figure 4**). This cell line is sensitive to stimuli at 2- 4 x 10^5 cells/ ml in 100 µl of serum free medium in a 96 well plate. For optimal sensitivity, BPS recommends using the provided protocols.

Application References

1. Weaver JR *et.al*. (2007) Characterization of the sequence and architectural constraints of the regulatory and core regions of the human interleukin-2 promoter. *Mol. Immunol*. **44**: 2813-2819.
2. Hughes CCW and Pober JS (1996) Transcriptional Regulation of the Interleukin-2 Gene in Normal Human Peripheral Blood T Cells. *J. Biol. Chem.* **271**: 5369-5377.

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Assay Protocol

A) Analysis of IL-2 Jurkat reporter activity in response to PMA/ionomycin

1. In a white opaque 96- well plate, seed cells at 2 - 4x10^4 cells/well (100 µl per well) in serum free RPMI medium. Cells should be growing at log phase at time of seeding.

2. Prepare fresh working solution of PMA (Fisher, Cat # BP685-1) at 10 ug/ml (from 1mg/ml stock in DMSO) and ionomycin (Fisher, Cat # BP25271) at 5 µg/ml (from 1 mg/ml stock in DMSO) in PBS.

3. Immediately treat 100 µl of cells with 10 µl of working solution of PMA and ionomycin for 24 hours at 37°C with 5% CO_2 (**Figure 2**). We used a starting concentration at 1 µg /ml for PMA and did a 3-fold serial dilution in PBS containing a constant level of ionomycin at 500 ng/ml (final concentration).

4. Add ONE-Step™ Luciferase Assay System (BPS Bioscience, Cat. No. 60690) to each well, according to recommended protocol.

5. Read luminescence using a luminometer. Normalize luminescence to wells that contain only medium to obtain the Relative Luminescence Units (RLUs).

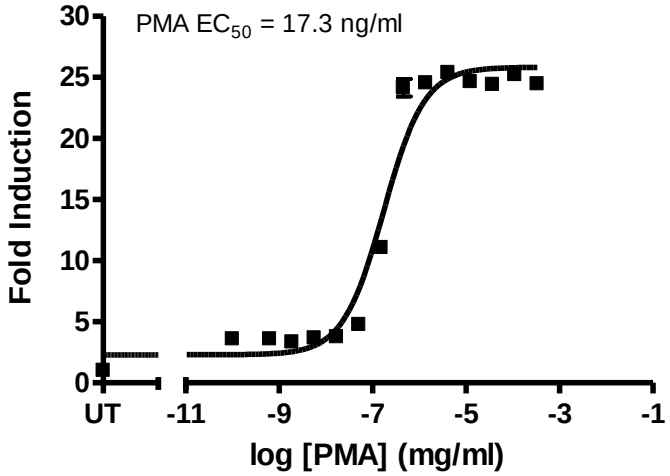

Figure 2. Response to PMA and Ionomycin Stimulation.
IL-2 Jurkat reporter cells were seeded at 3 x10^4 cells/well (100 µl per well) in serum free RPMI medium and treated with ionomycin and PMA. Error bar = standard deviation (SD), n=3.

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B) Analysis of IL-2 Jurkat reporter activity in response to anti-CD3 antibody

1. In a white opaque 96- well plate, seed cells at 1 - 4x10^4 cells/well (100 µl per well) in serum free RPMI medium overnight. Cells should be growing at log phase at time of seeding; do not use cells that have grown beyond 5 x 10^5 cells/ml in a T25 flask. Passage cells a day prior to seeding if cells are at high density.

2. The next day, immediately treat cells with anti-CD3 antibody for 24 hours at 37°C with 5% CO_2 (**Figure 3**). We used a starting concentration at 10 µg/ml, with subsequent three fold serial dilutions.

3. Add ONE-Step™ Luciferase Assay System (BPS Bioscience, Cat. No. 60690) to each well, according to recommended protocol.

4. Read luminescence using a luminometer. Normalize luminescence to wells that contain only medium to obtain the Relative Luminescence Units (RLUs).

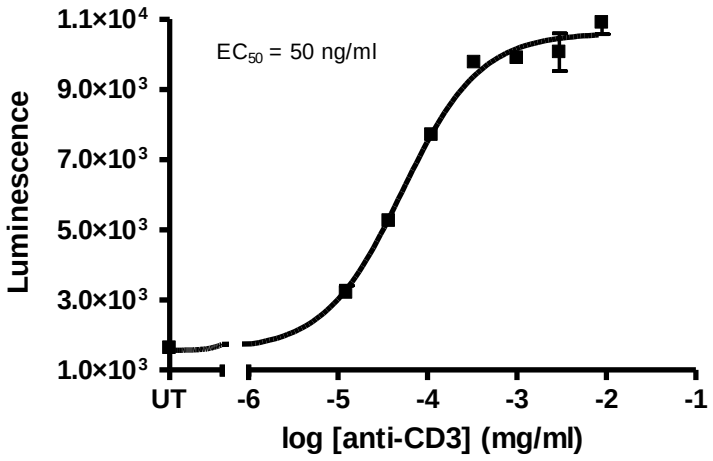

Figure 3. Response to Anti-human CD3 Antibody.
IL-2 Jurkat reporter cells were seeded at 1 x10^4 cells/well (100 µl per well) in serum free RPMI. Cells were treated with LEAF™ Purified anti-human CD3 antibody (clone OKT3, Biolegend Cat. No. 317325; BPS Cat. No.71274) over the indicated concentration range for 24 hours at 37°C with 5% CO_2. Error bar = standard deviation (SD), n=3.

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C) Analysis of IL-2 Jurkat reporter activity in response to TCR-Activator CHO Recombinant Cells.

1. In a white opaque 96- well plate, seed 1-4 x 10^4 TCR-Activator CHO cells in complete medium (10% FBS in F-12). Gently shake the plate to ensure cells are evenly distributed. Cells should be 60-80% confluent the next day. Do not use cells that are >90% confluent.

2. The next day, remove medium from each well, and add 2 - 4 x 10^4 IL-2 Jurkat reporter cells (100 µl per well) in serum free RPMI for ~24 hours at 37°C with 5% CO_2 (**Figure 4**).

3. Add ONE-Step™ Luciferase Assay System (BPS Bioscience, Cat. No. 60690) to each well, according to recommended protocol.

4. Read luminescence using a luminometer. Normalize luminescence to wells that contain only medium to obtain the Relative Luminescence Units (RLUs).

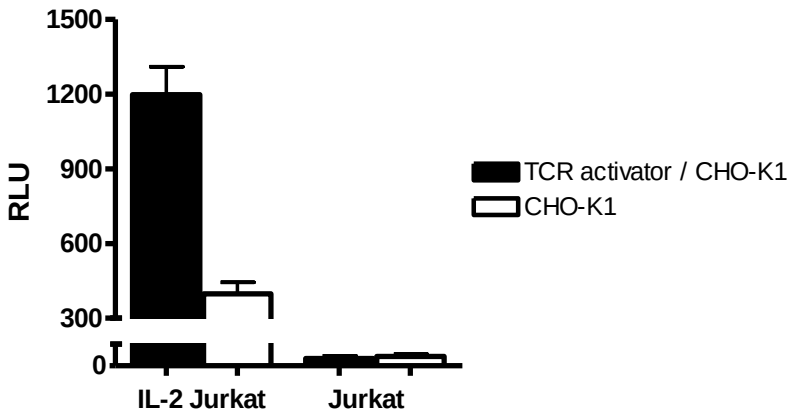

Figure 4. Response to TCR Activator - CHO Recombinant Cells.
4 x 10^4 TCR Activator - CHO Recombinant Cells (BPS Cat. No. 60539) or naïve CHO cells were co-cultured with IL-2 reporter Jurkat or naive Jurkat cells. Error bar = standard deviation (SD), n=9.

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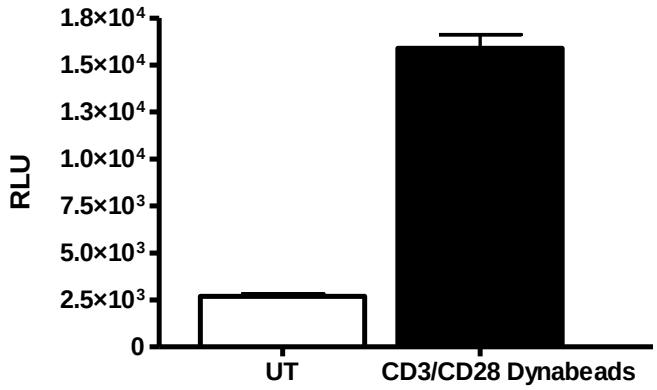

Figure 5. Response to Human T-Activator CD3/CD28 Dynabeads
IL-2 Jurkat cells were seeded on a white opaque 96-well plate at 2 x 10^4 cells/well (100 µl per well) in serum-free hybridoma medium. Cells were treated with anti-human CD3/CD28 Dynabeads (Thermo Fisher Cat. No. 1132D) at 4 µl per well for ~24 hours at 37°C with 5% CO_2, and compared to untreated cells. Error bar = standard deviation (SD), n=3.

Related Products

Product	**Cat. #**	**Size**
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Jurkat Cell Thawing Medium	60184	100 ml
TCR Activator/ CHO Cell Line	60539	2 vials
NFAT- Luc Jurkat Cell Line	60621	2 vials
Anti-CD3 Antibody	71274-2	100 ug

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