Description

This recombinant Jurkat cell line was engineered for conditional expression of firefly luciferase. It contains a firefly luciferase gene under the control of a human Interleukin-2 (IL-2) promoter stably integrated into the genome.

The IL-2 Luciferase Reporter Jurkat cell line is suitable to monitor the activation of the IL-2 promoter. This reporter cell line has been functionally validated using various agonists such as phorbol 12-myristate 13-acetate (PMA) in combination with ionomycin, an anti-human CD3 antibody, anti-human CD3/CD28 Dynabeads, and an anti-CD19/anti-CD3 bispecific antibody. It has also been validated in a co-culture assay with our TCR activator CHO cell line.

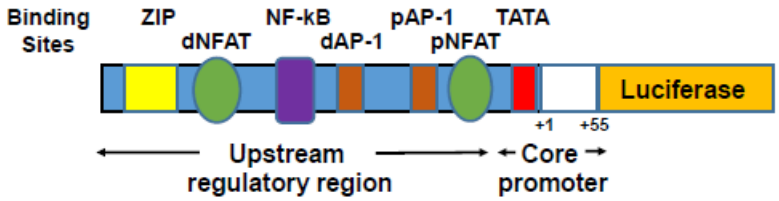

Figure 1: Illustration of the IL-2 promoter region with representative transcription factor binding sites.

Background

Interleukin-2 (IL-2) is a key cytokine regulating the proliferation and differentiation of T cells into effector T cells. Gene expression of IL-2 is induced by activation of the TCR (T Cell Receptor) upon binding to MHC/peptide complexes. TCR activation results in the stimulation of transcription factors AP-1 (Activator protein 1), NFκB (Nuclear factor kappa-light-chain-enhancer of activated B cells) and NFAT (Nuclear factor of activated T-cells), which bind to canonical response elements in the promoter of IL-2. Co-stimulation of CD28 synergizes with the TCR to promote optimal IL-2 expression and secretion.

Induction of the luciferase reporter gene under the control of the IL-2 promoter is an excellent proxy for TCR activation in this Jurkat cell line.

Application

- Screen for agonists of T cell receptors.
- Quantify T cell activation through T cell receptor (TCR).
- Analyze the functional activity of bispecific antibodies, such as Bispecific T cell Engagers.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

Jurkat (clone E6-1), human T lymphoblast, suspension

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2B	BPS Bioscience #79530

Materials Used in the Cellular Assays

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Bovine Serum Albumin (BSA, protease free)	Sigma #A4919
Phorbol 12-myristate 13-acetate (PMA)	Fisher Scientific #BP685-1
Ionomycin	Fisher Scientific #BP25271
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
TCR Activator - CHO Recombinant Cells	BPS Bioscience #60539
Dynabeads™ Human T-Activator CD3/CD28	Thermo Fisher #11131D
Raji Cells	ATCC #CCL-86
Anti-CD19 x anti-CD3 bispecific antibody	BPS Bioscience #100441
Cbl-b-IN-1	MedChemExpress #HY-136339
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated white, clear-bottom plate	
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 medium supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Growth Medium 2B (BPS Bioscience #79530):
RPMI 1640 medium supplemented with 10% FBS and 1% Penicillin/Streptomycin supplemented with 1 mg/ml Geneticin.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Thaw Medium 3 (BPS Bioscience #60186):
F-12K medium supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Assay Medium 2B (BPS Bioscience #79619):
RPMI 1640 medium supplemented with 1% Penicillin/Streptomycin

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 2 **(no Geneticin)**.
   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium **2 (no Geneticin)**.

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium **2 (no Geneticin)**, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium **2B (contains Geneticin)**.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, at no less than 0.2 x 10^6 cells/ml of Growth Medium **2B (contains Geneticin)**. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

3. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Functional characterization of IL-2 Luciferase Reporter Jurkat Cell Line

The following assays were designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately. **Each assay should be performed at least in triplicates.**

a. IL-2 Luciferase Reporter Jurkat cell activation by PMA and Ionomycin

Assay Medium: Assay Medium 2B

1. Seed IL-2 Luciferase Reporter Jurkat cells in 100 µl/well of Assay Medium 2B at a density of ~30,000 cells per well into a white, clear-bottom 96-well plate. Cells should be growing at log phase at time of seeding. Keep three wells without cells for determination of background luminescence.

2. Prepare a three-fold serial dilution of PMA at concentrations 11-fold higher than the desired final concentrations in Assay Medium 2B. To obtain the EC_{50} dose curve shown below, we used a PMA dose range of 0.001 to 1,000 ng/ml in the presence of 500 ng/ml Ionomycin.

   - Add 10 µl of diluted PMA/Ionomycin to each well (final volume is 110 µl).

   - Add 10 µl of Assay Medium 2B with the same concentration of DMSO as was used for the agonist to the "Unstimulated control" wells.

   - Add 110 µl of Assay Medium 2B to the cell-free control wells (for determining background luminescence).

3. Incubate the cells at 37°C in a CO_2 incubator for ~24 hours.

4. The next day, perform the luciferase assay using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Add 110 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 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. The fold induction of IL-2 luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{luminescence\ of\ stimulated\ cells - avg.background}{avg.luminescence\ of\ unstimulated\ cells - avg.background}$$

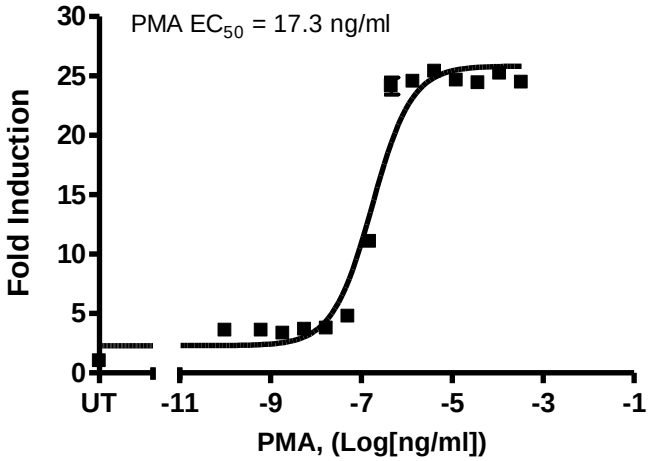

Figure 2: IL-2 Luciferase Reporter Jurkat cell response to PMA + Ionomycin
Cells were treated with increasing concentrations of PMA plus a fixed concentration of 500 ng/ml Ionomycin for 24 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression compared to the "untreated (UT)" control.

b. IL-2 Luciferase Reporter Jurkat cell activation by anti-CD3 antibody

Assay Medium: Assay Medium 2B + 0.1% BSA (protease free)

1. Seed IL-2 Luciferase Reporter Jurkat cells in 50 µl of assay medium at a density of ~70,000 cells per well into a white, clear-bottom 96- well plate. Cells should be growing at log phase at time of seeding. Keep three wells without cells for determination of background luminescence. Incubate at 37°C in a CO_2 incubator overnight (~18 hours).

2. The next day, prepare a three-fold serial dilution of anti-CD3 antibody at concentrations 2-fold higher than the final desired concentrations in Assay Medium 2B (containing BSA). For the EC_{50} dose curve shown below, we used a final dose range of 0.0001 to 10 µg/ml.

   - Add 50 µl of diluted anti-CD3 antibody to each well (final volume is 100 µl).
   - Add 50 µl of assay medium to the "Unstimulated control" wells.
   - Add 100 µl of assay medium to the cell-free control wells (for determining background luminescence).

3. Incubate the cells at 37°C in a CO2 incubator for ~24 hours.

4. The next day, perform the luciferase assay using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 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. The fold induction of IL-2 luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{luminescence\ of\ stimulated\ cells - avg.background}{avg.luminescence\ of\ unstimulated\ cells - avg.background}$$

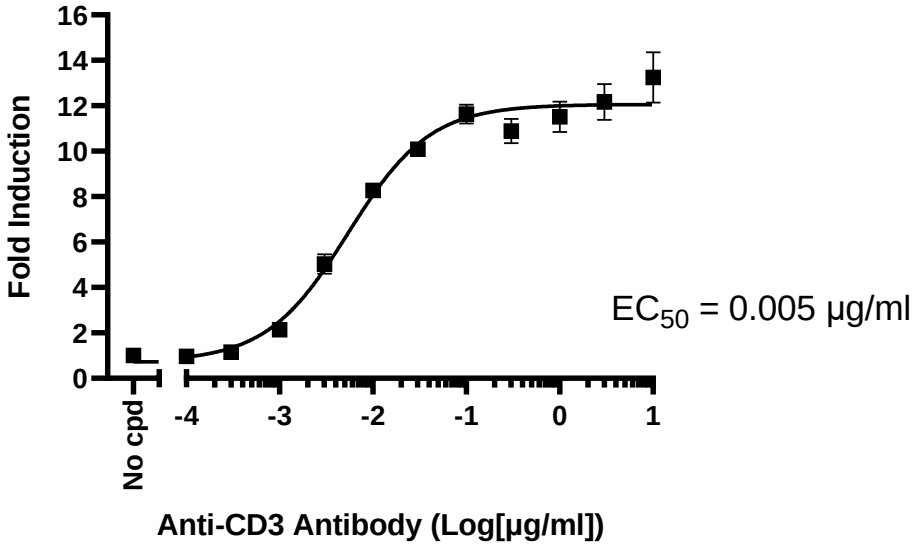

Figure 3: IL-2 Luciferase Reporter Jurkat cells response to anti-CD3 antibody.
Cells were treated with increasing concentrations of anti-CD3 antibody (clone OKT3, BPS Bioscience #71274) for 24 hours at 37°C with 5% CO_2. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are expressed as fold induction compared to the "No compound" control.

c. IL-2 Luciferase Reporter Jurkat cell activation in a co-culture assay with TCR activator CHO cells

Assay Medium: Assay Medium 2B

1. Seed TCR activator CHO cells (BPS Bioscience #60539) in 100 µl of Thaw Medium 3 at a density of ~40,000 cells per well into a white, clear-bottom 96-well plate. Leave three wells without cells for determination of background luminescence. Cells should be 60-80% confluent the next day. Do not use cells that are >90% confluent.

2. The next day, remove Thaw Medium 3 from each well and add IL-2 Luciferase Reporter Jurkat cells in 100 µl Assay Medium 2B at a density of ~40,000 cells per well.

3. Incubate the cells at 37°C in a CO_2 incubator for ~24 hours.

4. The next day, perform the luciferase assay using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.

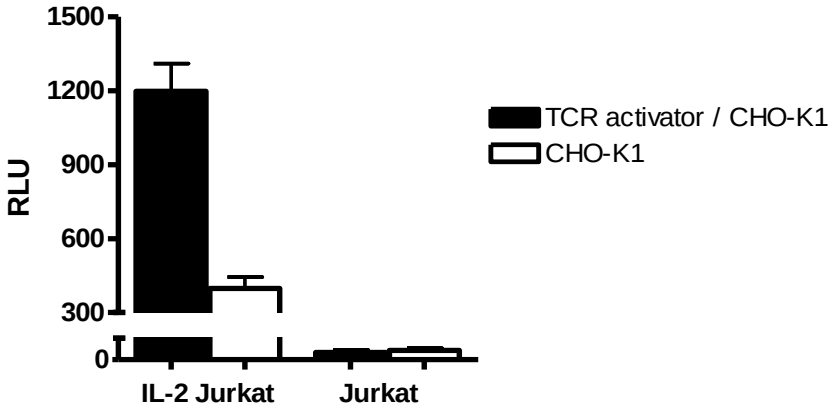

Figure 4: IL-2 Luciferase Reporter Jurkat cells response to TCR Activator CHO Recombinant Cells.
IL-2 Luciferase Reporter Jurkat cells and control Jurkat cells were co-cultured with TCR activator CHO cells (BPS Bioscience #60539) or parental CHO cells for ~24 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are expressed as raw luminescence signal.

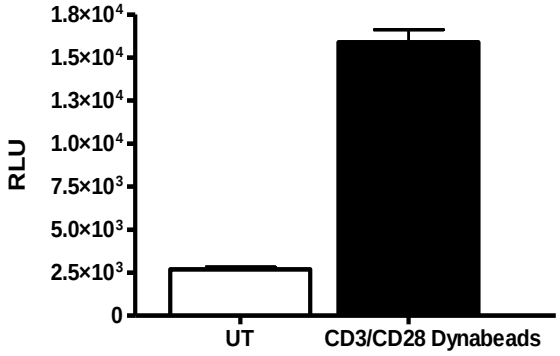

Figure 5. IL-2 Luciferase Reporter Jurkat cells response to CD3/CD28 Dynabeads.
IL-2 Luciferase Reporter Jurkat cells were seeded on a white opaque 96-well plate at 2 x 10^4 cells/well (100 µl per well) in serum-free hybridoma medium. Cells were treated with anti-human CD3/CD28 Dynabeads (Thermo Fisher #1132D) at 4 µl per well for ~24 hours at 37°C with 5% CO_2. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are expressed as raw luciferase signal. UT: untreated control. Error bar = standard deviation (SD), n=3.

d. IL-2 Luciferase Reporter Jurkat cell activation by anti-CD19/anti-CD3 bispecific antibody (Blinatumomab) in a co-culture assay with CD19-positive Raji cells

Assay Medium: Thaw Medium 2

1. Seed CD19-positive Raji cells in 25 µl of Thaw Medium 2 at a density of 30,000 cells per well in a white, clear-bottom 96-well plate.

   Note: it is important to optimize the Raji cell number seeded per well. The optimal number of cells can vary based on the passage number and culture density prior to seeding.

2. To the Raji cells, add IL-2 Luciferase Reporter Jurkat cells in 25 µl Thaw Medium 2 at a density of 30,000 cells per well.

3. Prepare a three-fold serial dilution of anti-CD19/anti-CD3 bispecific antibody (Blinatumomab) at concentrations 2-fold higher than the desired final concentrations in Thaw Medium 2. For the EC_{50} dose curve shown below, we used a final dose range of 0.001 to 100 ng/ml.

   - Add 50 µl of diluted bispecific antibody to each well (final volume is 100 µl).
   - Add 50 µl of Thaw Medium 2 to the "Unstimulated control" wells.
   - Add 100 µl of Thaw Medium 2 to the cell-free control wells (for determining background luminescence).

4. Incubate at 37°C in a CO_2 incubator overnight.

5. The next day, perform the luciferase assay using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.

6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of IL-2 luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{luminescence\ of\ stimulated\ cells - avg.background}{avg.luminescence\ of\ unstimulated\ cells - avg.background}$$

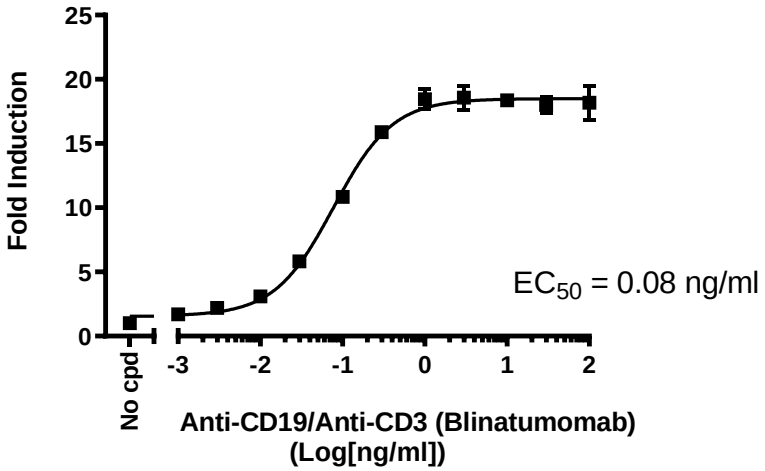

Figure 6: IL-2 Reporter Jurkat cell response to anti-CD19/anti-CD3 bispecific antibody in the presence of CD19-positive Raji cells.
IL-2 Reporter Jurkat cells were co-cultured with CD19-positive Raji cells and treated with increasing concentrations of anti-CD19/anti-CD3 bispecific antibody (BPS Bioscience #100441) for 24 hours at 37°C with 5% CO_2. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are expressed as fold induction compared to the "No compound" control.

e. IL-2 Luciferase Reporter Jurkat cell activation using an inhibitor of Cbl-b

CBL-B is an E3 ligase acting as a negative regulator of T cell activation. Adding a CBL-B inhibitor to Jurkat T cells results in an increase in IL-2 promoter activity because the brake imposed by CBL-B on constitutive TCR signaling is removed.

Assay Medium: Thaw Medium 2

1. Seed IL-2 Luciferase Reporter Jurkat cells in 50 µl of Thaw Medium 2 at a density of ~70,000 cells per well into a white, clear-bottom 96- well plate. Keep three wells without cells for determination of background luminescence. Each condition should be performed in triplicate.

2. Prepare a three-fold serial dilution of inhibitor Cbl-b-IN-1 at concentrations 2-fold higher than the final desired concentrations in Thaw Medium 2. To obtain the EC_{50} dose curve shown below, we used a final dose range of 0.0001 to 10 µM.

   - Add 50 µl of diluted Cbl-b-IN-1 to each well (final volume is 100 µl).

   - Add 50 µl of Thaw Medium 2 to the "Unstimulated control" wells.

   - Add 100 µl of Thaw Medium 2 to the cell-free control wells (for determining background luminescence).

3. Incubate at 37°C in a CO_2 incubator overnight.

4. The next day, perform the luciferase assay using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 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. The fold induction of IL-2 luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{luminescence\ of\ stimulated\ cells - avg.background}{avg.luminescence\ of\ unstimulated\ cells - avg.background}$$

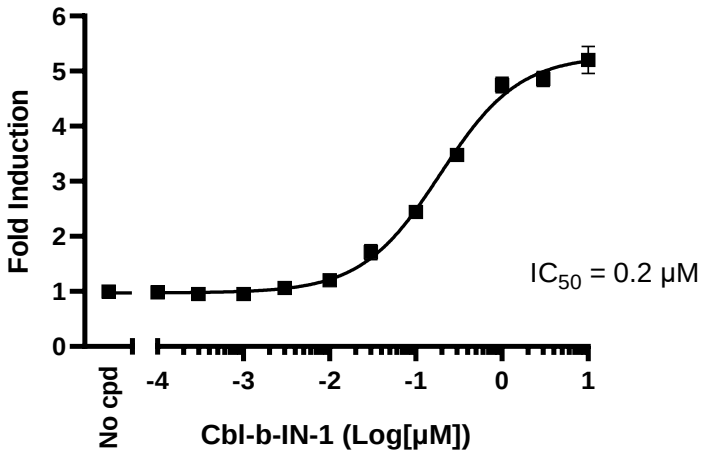

Figure 7: IL-2 Reporter Jurkat cell response to Cbl-b-IN-1.
Cells were treated with increasing concentrations of Cbl-b-IN-1 (MedChemExpress #HY-136339) for 24 hours at 37°C with 5% CO_2. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are expressed as fold induction compared to the "No compound" control.

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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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
IL-15 Responsive Luciferase Reporter Cell Line	78402	2 vials
NFAT Luc Jurkat Cell Line	60621	2 vials
TCR Activator - Raji Cell Line	60556	2 vials
Firefly Luciferase Raji Cell Line	78622	2 vials
CTLA4 / IL-2 Reporter - Jurkat Recombinant Cell Line	79525	2 vials