Description

The forkhead box P3 (Foxp3, also known as scurfin) Luciferase Reporter Jurkat cell line is designed for monitoring the transcriptional activity of the Foxp3 promoter and testing inhibitors that target Foxp3-inducing signaling pathways. It contains a firefly luciferase gene under the control of a human Foxp3 promoter and an enhancer-like conserved noncoding sequence upstream of the Foxp3 promoter.

This reporter cell line has been tested and validated using phorbol 12-myristate 13-acetate (PMA) in combination with ionomycin. It can be used to measure T cell activation mediated by Dynabeads T-Activator CD3/CD28.

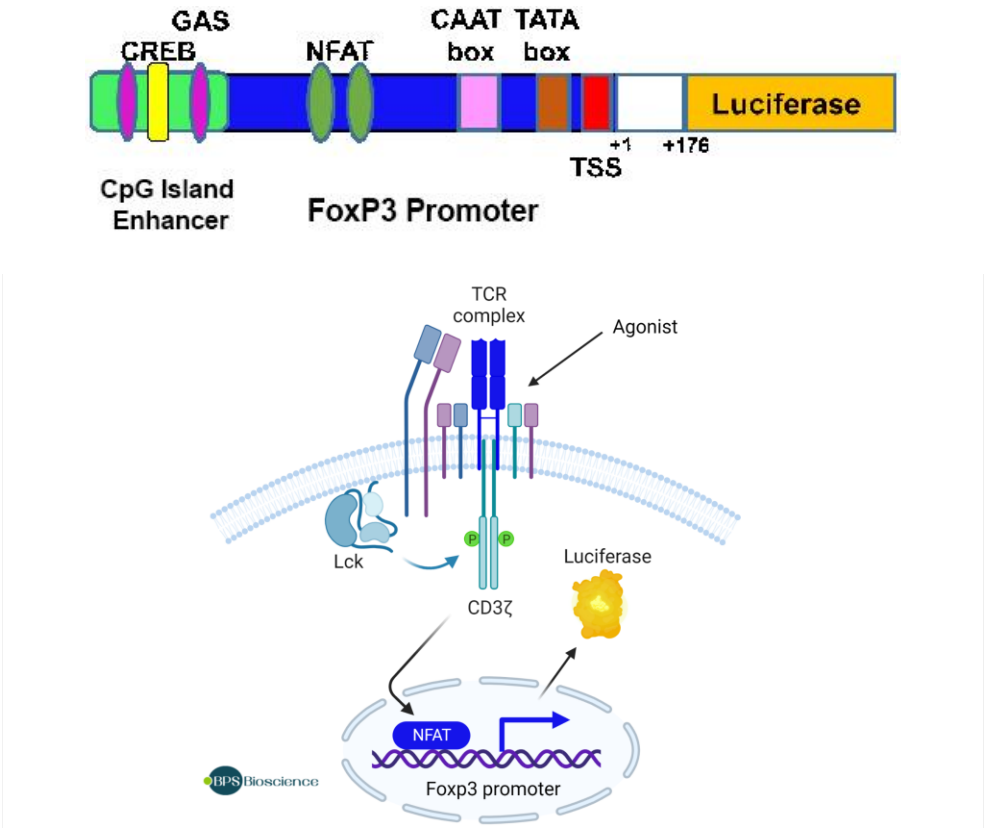

Figure 1: Illustration of the Foxp3 promoter region with representative transcription factor binding sites and enhancer regions, and illustration of the cell-based assay principle (created with BioRender.com).

Background

Foxp3, belonging to the forkhead family, is a master transcriptional regulator that is expressed exclusively in regulatory T cells, a subset of $CD4^+$ T cells that downregulate immune responses. Regulation of Foxp3 is critical for maintaining immunological tolerance, and over-expression of Foxp3 is known to suppress effector T cell activation. The promoter of the gene encoding Foxp3 contains sequence binding elements recognized by transcription factors that coordinate the expression of Foxp3, including *CREB* (CAMP Response Element Binding), *GAS* (Gamma interferon activation site) and *NFAT* (nuclear factors of activated T cells) binding elements.

Application

Screen for compounds that affect transcription factors that regulate the expression of Foxp3 in a cellular context.

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 Required for Cellular Assay

Name	Ordering Information
Assay Medium 2E	BPS Bioscience #78826
Phorbol 12-myristate 13-acetate (PMA)	LC Laboratories #P1680
Ionomycin	Sigma #I3909
Dynabeads™ Human T-Activator CD3/CD28	Thermo Fisher #11131D
Cyclosporin A	Sigma #30024
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	
96-well tissue culture-treated white clear-bottom assay plate	

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℃ 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, 1% Penicillin/Streptomycin.

Growth Medium 2B (BPS Bioscience #79530):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin and 1 mg/ml G418.

Media Required for Functional Cellular Assay

Assay Medium 2E (BPS Bioscience #78826):
RPMI 1640 medium supplemented with 0.5% FBS, 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 G418).**
   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 G418).**

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 G418)**, 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 G418).**

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 G418).** 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. Validation Data

- The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- Perform the experiment in triplicates.
- This assay uses a reduced serum medium.

Assay Medium 2E (BPS Bioscience #78826):
RPMI 1640 medium supplemented with 0.5% FBS, 1% Penicillin/Streptomycin.

a. Activation of luciferase by small molecule agonists in Foxp3 Luciferase Reporter Jurkat cell

1. Seed Foxp3 Reporter Jurkat cells in 75 ml of Assay Medium at a density of 40,000 cells per well into a white, clear-bottom 96-well plate. Cells should be growing at log phase at time of seeding. Keep a few wells without cells for determination of background luminescence.

2. Prepare a mixture of PMA and ionomycin in Assay Medium at concentrations 4-fold higher than the final desired concentration (Final [PMA] = 1 nM; Final [Ionomycin] = 225 nM).

3. Add 25 ml of the PMA and ionomycin mixture to each well.

4. Add 25 ml of Assay Medium with the same concentration of DMSO as was used for the PMA/ionomycin dilution to the "untreated" control wells.

5. Add 100 ml of Assay Medium with DMSO to the cell-free control wells to determine background luminescence.

6. Incubate the cells at 37°C in a 5% CO_2 incubator for ~24 hours.

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

8. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of Foxp3 luciferase reporter expression is the background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ Induction = \frac{Lumin.\ of\ stimulated - background}{Lumin.\ of\ unstimulated - background}$$

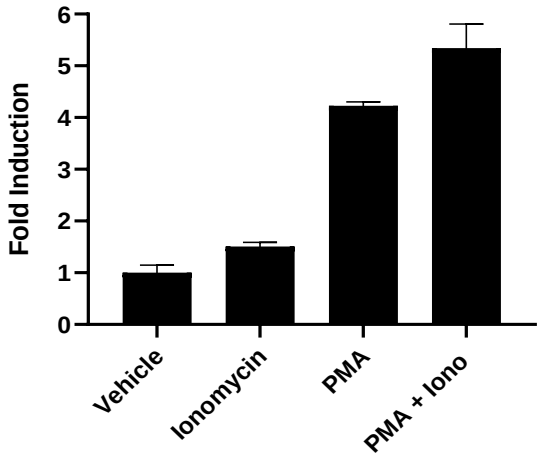

Figure 2: Activation of Foxp3 Luciferase Reporter Jurkat cell by PMA and Ionomycin.
Cells were treated with 225 nM ionomycin, 1 nM PMA, or a mixture of 225 nM ionomycin + 1 nM PMA for ~18 hours. As expected, the combination of ionomycin and PMA produced the highest luciferase signal.

b. Activation of Foxp3 Luciferase Reporter Jurkat cells by CD3/CD28 Dynabeads™

1. Seed Foxp3 Luciferase Reporter Jurkat cells in 50 ml of Assay Medium at a density of 40,000 cells per well into a white, clear-bottom 96-well plate. Cells should be growing at log phase at time of seeding. Keep a few wells without cells for determination of background luminescence.

2. Prepare CD3/CD28 Dynabeads™ according to the Thermo Fisher #11131D protocol. Prepare Dynabead™ dilutions in Assay Medium at desired bead-to-cell ratio.

3. Add 50 ml of Dynabead™ solutions to the “treated wells”.

4. Add 50 ml of Assay Medium to the “untreated” control wells.

5. Add 100 ml of Assay Medium to the cell-free control wells to determine background luminescence.

6. Incubate the cells at 37°C in a 5% CO_2 incubator for ~18 hours.

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

8. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of Foxp3 luciferase reporter expression is the background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ Induction = \frac{Lumin.of\ stimulated - background}{Lumin.of\ unstimulated - background}$$

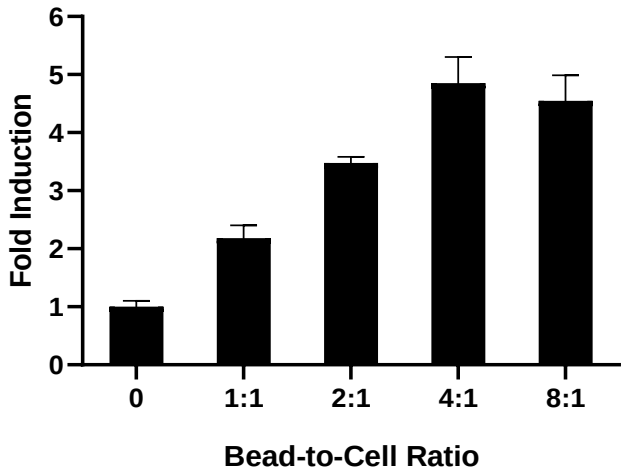

Figure 3: Foxp3 Reporter Jurkat response to TCR activator CD3/CD28 Dynabeads™.
Cells were incubated with the indicated bead-to-cell ratios of Human T-activator CD3/CD28 Dynabeads™ (Thermo Fisher #11131D). Dynabeads are a trademark of Thermo Fisher Scientific.

c. Inhibition of PMA/Ionomycin-induced Foxp3 activity by Cyclosporin A in Foxp3 Luciferase Reporter Jurkat cells.

The effect of the inhibitor is measured against activation by PMA/ionomycin. The experiment requires a cell-free control for determining background signal, un "untreated" control without PMA/ionomycin, and a PMA/ionomycin-treated condition without inhibitor for the "Positive control".

1. Seed Foxp3 Luciferase Reporter Jurkat cells in 50 ml of Assay Medium at a density of 40,000 cells per well into a white, clear-bottom 96-well plate. Cells should be growing at log phase at time of seeding. Keep a few wells without cells for determination of background luminescence.

2. Prepare a three-fold serial dilution of Cyclosporin A (the test inhibitor) in Assay Medium at concentrations 4-fold higher than the final desired concentrations.

3. Add 25 ml of serially diluted inhibitor to each well.

4. Add 25 ml of Assay Medium with the same concentration of DMSO as was used for the Cyclosporin A dilution to the "untreated" control wells.

5. Prepare a mixture of PMA and ionomycin in Assay Medium at concentrations 4-fold higher than the final desired concentration (Final [PMA] = 1 nM; Final [Ionomycin] = 225 nM).

6. Add 25 ml of PMA/ionomycin mixture to each well.

7. Add 25 ml of Assay Medium with the same concentration of DMSO as was used for the PMA/ionomycin mixture to the "unstimulated" control wells.

8. Add 100 ml of Assay Medium with DMSO to the cell-free control wells to determine background luminescence.

9. Incubate the cells at 37°C in a 5% CO_2 incubator for ~24 hours.

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

11. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent luminescence of Foxp3 luciferase reporter expression is the background-subtracted luminescence of the treated wells divided by the average background-subtracted luminescence of the untreated control wells x 100%.

$$Percent\ Luminescence = \left(\frac{Lumin.of\ inhibitor\ treated\ cells - background}{Lumin.of\ untreated\ cells - background}\right) x100$$

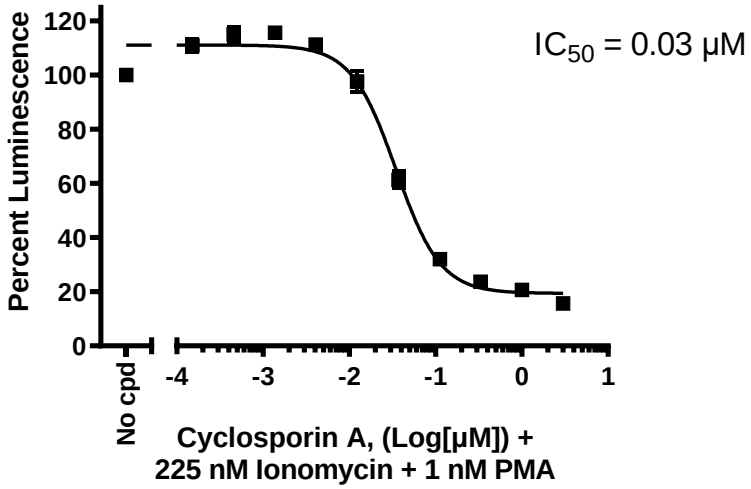

Figure 4: Inhibition of PMA/Ionomycin-induced Foxp3 Luciferase Reporter activity by Cyclosporin A.
Cells were treated with increasing concentrations of Cyclosporin A and 225 nM ionomycin + 1 nM PMA for 24 hours. Cyclosporin A inhibited PMA/ionomycin-induced Foxp3 luciferase reporter activity.

References

1. Tone Y et.al. (2008) *Nat Immunology*. 9: 194-202
2. Liu R *et.al*. (2015) *Cancer Res*. 75: 1703-1713
3. Soligo M. *et al.* (2011) *Eur J. Immunology*. 41: 503-513

Vector

Human Foxp3 promoter-Luciferase was cloned into the MCS of pCDNA3.1™ (+) vector (Invitrogen #V79020).

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

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*
TCR Activator Raji Cell Line	60556	2 vials
TCR Activator/FcGR2B CHO Cell Line	78436	2 vials
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	78556	2 vials
TCR CRISPR/Cas9 Lentivirus (Integrating)	78055	500 µl x 2