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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Foxp3 Reporter (Luc) - Jurkat Recombinant Cell Line
Catalog # 60628

Description
Human Foxp3 luciferase reporter construct is stably integrated into the genome of Jurkat T-cells. The firefly luciferase gene is controlled by a human Foxp3 promoter and an enhancer-like conserved noncoding sequence upstream of the Foxp3 promoter.

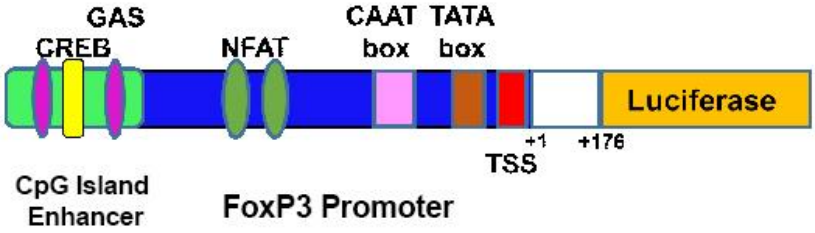

Figure 1. Illustration of Foxp3 promoter region with representative transcription factor binding sites and enhancer regions.

Background
Foxp3, belonging to the forkhead family, is a master transcription factor that expresses exclusively in regulatory T cells, a subset of CD4+ T cells. Regulation of Foxp3 is critical for maintaining immunological tolerance. Over-expression of Foxp3 is known to suppress effector T cell activation.

Host Cell
Jurkat (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 Media
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) and 1 mg/ml G418 (Thermo Fisher, Cat. #11811031).

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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 **(no G418)**. 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 conical tube with Thaw Medium 2 **(no G418)** and rock the tube the tube gently. Centrifuge the cells at 200 x g for 3 minutes. Re-suspend the cells in 6 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 reached 80% confluency, 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 **(contains G418)**; gently pipette up and down to dissociate cell clumps. *This cell line is "clumpy" (indicates healthy growth). It is highly advised to dissociate the clumps by gentle pipetting during passaging or seeding for assays.* Dispense 2-4 ml of the cell suspension into a new T-75 flask containing pre-warmed 15 ml Growth Medium 2B. Incubate cells in a humidified 37°C incubator with 5% CO_2. Freeze cells in freezing medium (10% DMSO in FBS) when cells reach 80% confluency. Cells have been demonstrated to be stable for at least 15 passages; BPS Bioscience 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. #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Cat. #LT07-518) was used as a positive control.

Application

The Foxp3 promoter Jurkat reporter cell line is suitable for monitoring the transcription activity of Foxp3 in response to stimulant, and establishing cell-based screens for inhibitors that target specific Foxp3-stimulating molecules. This reporter cell line has been tested and validated using phorbol 12-myristate 13-acetate (PMA) with ionomycin (**Figure 2**). BPS Bioscience does not recommend starving the cells overnight in serum-free medium prior to stimulation.

Materials Required but Not Supplied for Cell Culture

- Thaw Medium 2 (BPS Bioscience #60184)
- Growth Medium 2B (BPS Bioscience #79530)

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Materials Required but Not Supplied for Cellular Assay

- Assay Medium 2B (BPS Bioscience #79619)
- PMA (Fisher, Cat # BP685-1)
- Ionomycin (Fisher, Cat # BP25271)
- ONE-Step™ Luciferase Assay System (BPS Bioscience #60690)
- Luminometer
- 96-well tissue culture-treated white clear-bottom assay plate

Application References

1. Mantel, P.Y., *et al.* (2006) Molecular Mechanisms Underlying FOXP3 induction in Human T cells. *J. Immunology* **176(6):**3593-602.

Assay Protocol

1. In a white opaque 96- well plate, seed cells at ~1 x10^4 cells/well (100 µl per well) in Assay Medium 2B (BPS #79619)

   Assay Medium 2B (BPS #79619): RPMI 1640 medium (Thermo Fisher, Cat. #A1049101) supplemented with 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

   Cells should be growing at log phase at time of seeding. Using cells from over-confluent culture (ie. stationary growth) will significantly reduce signal output.

2. Prepare fresh working solution of PMA (Fisher, Cat # BP685-1) at 100 ng/ml (from 100 µg/ml stock in DMSO) and ionomycin (Fisher, Cat # BP25271) at 1.67 µg/ml (from 1 mg/ml stock in DMSO) in PBS.

3. Immediately treat cells with 10 µl of working solution of PMA (ie. 10 ng/ml, final concentration) and ionomycin (167 ng/ml, final concentration) for 24 hours at 37°C with 5% CO_2 (**Figure 2**).For maximum signal, the final concentration should be 167 ng/ml ionomycin and 0.15-30 ng/ml of PMA in 100 µL of medium (**Figure 3**).

4. Add ONE-Step™ Luciferase Assay System (BPS Bioscience, Cat. #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).

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Quality Assurance and Functional Analysis

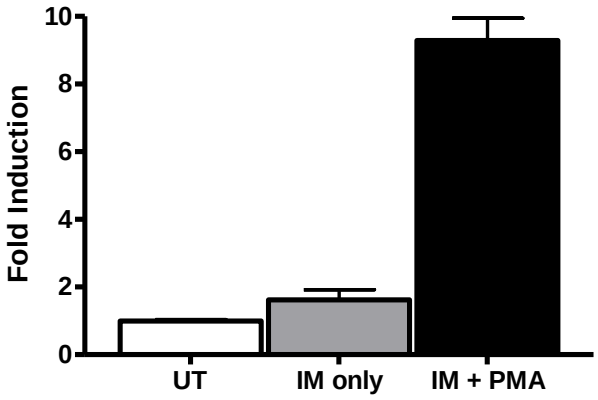
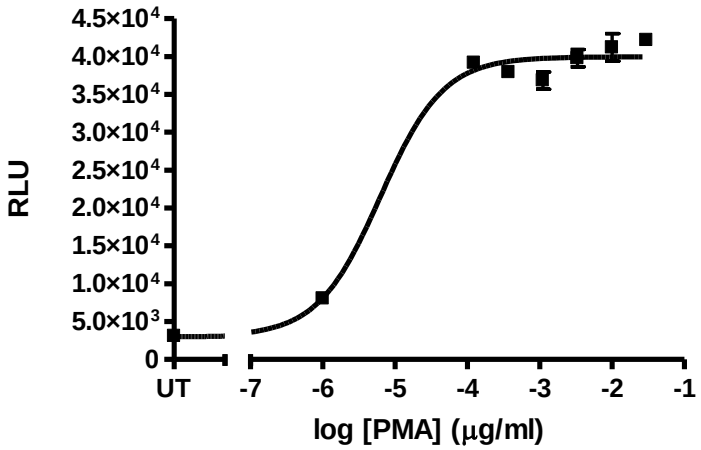


Figure 2. Foxp3 Jurkat reporter responses to a combination of PMA and ionomycin. Foxp3 Jurkat reporter cells were seeded on a white opaque 96- well plate at approximately 1 x104 cells/well (100 µl per well) in serum free RPMI medium. Cells were treated with 167 ng/ml ionomycin (IM only), with 167 ng/ml IM and 10 ng/ml PMA (IM+PMA) or untreated (UT) at 37°C with 5% CO2. Fold induction = relative luminescence normalized to untreated cells; n=4.



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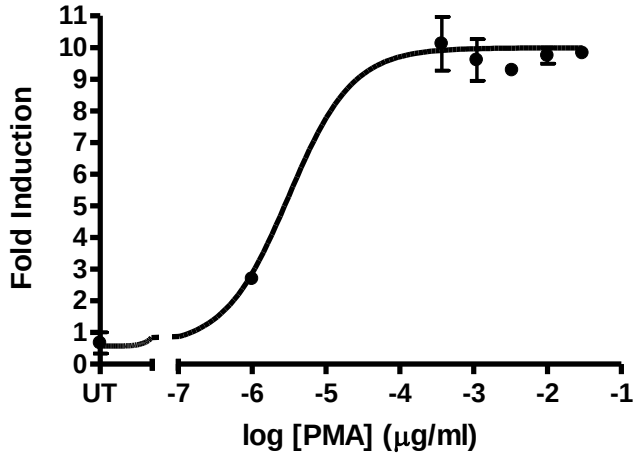

Figure 3. Foxp3 Jurkat reporter activity in response to PMA and ionomycin.
Approximately 1 x10^4 cells/well (100 µl per well) in serum free RPMI medium were untreated (UT) or treated with 167ng/ml of ionomycin with 0- 30 ng/ml of PMA for 24h. (Top) Clone 1D8; RLU = relative luminescence normalized to RPMI. (Bottom) Clone 1C9; Fold induction = RLU of treated cells with respect to untreated cells; n=3. Error bar = standard deviation (SD).

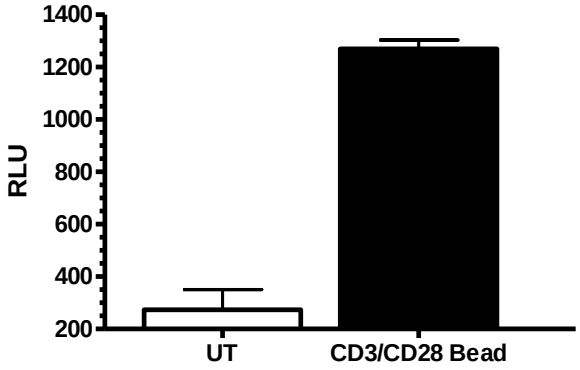

Figure 4. TCR activator CD3/CD28 stimulation.
Approximately 8000 cells/well in serum-free medium were treated with 5µL of Human T-Activator CD3/CD28 Dynabead (Thermo Fisher, Cat. No. 11161D) for 24 hours.

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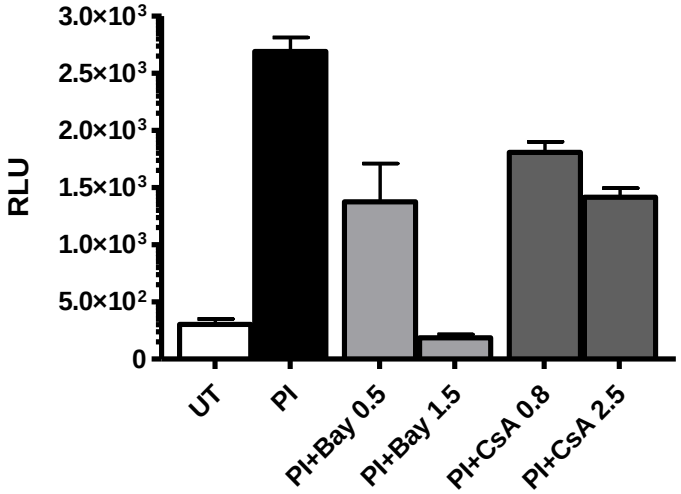

Figure 5. Inhibition of Foxp3 reporter by BAY11-7082 and Cyclosporin A.
Approximately 8000 cells/ well in SF- medium were left untreated (UT), treated with 30ng/ml of PMA and 167ng/ml Ionomycin (PI), PI with 0.5 or 1.5 µM Bay 11-7082 (Bay; Sigma Cat. No. B5556) (PI + Bay), or PI with 0.8 or 2.5 µM Cyclosporin A (CsA; Sigma Cat. No. 30024) for 24 hours. n =3. RLU = Relative Luminescence Unit.

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

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

Related Products

Product	**Cat. #**	**Size**
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
ONE-Step™ Luciferase Assay System	60690-3	1 L
Thaw Medium 2	60184	100 ml
Growth Medium 2B	79530	500 ml
Assay Medium 2B	79619	100 ml