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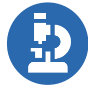 Laborgeräte & Service

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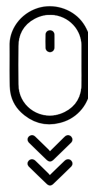

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SZABO-SCANDIC HandelsgmbH

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 West, Suite B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

NFAT Reporter (Luc) – Jurkat Cell line

Catalog #: 60621

Background

The nuclear factor of activator T cells (NFAT) family of transcription factors plays an important role in immune response. T cell activation through the T cell synapse results in calcium influx. Increased intracellular calcium levels activate the calcium-sensitive phosphatase, calcineurin, which rapidly dephosphorylates the serine-rich region (SRR) and SP-repeats in the amino termini of NFAT proteins. This results in a conformational change that exposes a nuclear localization signal promoting NFAT nuclear import. In the nucleus, NFAT proteins cooperate with other proteins to bind to DNA.

Product Description

The *NFAT Reporter – Jurkat Cell Line* contains a firefly luciferase gene under the control of the NFAT response element stably integrated into Jurkat cells. This cell line has been validated for response to thapsigargin, ionomycin, phorbol 12-myristate 13-acetate (PMA), and anti-CD3 Ab, and has also been validated for T cell activation through a variety of TCR activators: including TCR activator (anti-CD3ε scFv)/CHO cells (BPS #60539); CD3xCD19 Bispecific Antibody (BiTE) or Blinatumomab in the presence of CD19+ Raji cells; and anti-BCMA-CAR in the presence of BCMA/CHO cells. The reporter cell line is designed to monitor T cell activation as well as inhibition through various immune checkpoint inhibitors; it can be used as a control or parental cell line to co-express various immune checkpoint inhibitors, such as PD1. It can also be used to assess the activity of Bispecific antibodies (e.g. BiTE) and CAR

Application

- Monitor intracellular calcium levels.
- Screen for activators or inhibitors of PKC/ Ca^{2+} pathway.
- Screen for agonists or antagonists of T cell receptors and co-inhibitors
- Control for immune checkpoint NFAT reporter cell lines.
- Determine T cell activation through T cell receptor (TCR) or CAR (Chimeric Antigen Receptor)
- Analysis the functional activity of Bispecific antibodies, (e.g. BiTE: Bispecific T cell Engager)
-

Format

Each vial contains ~$2x10^6$ cells in 1 ml of 10% DMSO.

Storage

Immediately upon receipt, store in liquid nitrogen.

Culture conditions

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

Growth Medium 2B (BPS Cat. #79530): Thaw Medium 2 (BPS Cat. #60184) and 1 mg/ml of Geneticin (Life Technologies #11811031).

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

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer to a tube containing 10 ml of Thaw Medium 2 (**no Geneticin)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2 **(no Geneticin).** Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. At first passage, switch to Growth Medium 2B **(contains geneticin)**. Cells should be split before they reach $2x10^6$ cells/ml. To passage the cells, dilute cell suspension into new culture vessels at no less than 0.1 $x10^6$ cells/ml. Subcultivation ratio: 1:10 to 1:20 weekly. Freeze down cells in FBS plus 10% DMSO.

Mycoplasma testing
The cell line has been screened using the PCR-based VenorGeM® Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Functional Validation and Assay Performance
The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.

Materials Required but Not Supplied

- PMA (LC Laboratories #P1680): Prepare stock solution in DMSO.
- Ionomycin (Sigma #I3909): Prepare stock solution in DMSO.
- Thapsigargin (Sigma #T9033): Prepare stock solution in DMSO.
- Assay medium: Thaw Medium 2 (BPS Cat. #60184)
- Growth Medium 2B (BPS Cat. #79530)
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- One-Step™ luciferase assay system (BPS Bioscience #60690) for measuring firefly luciferase activity
- Luminometer

A. NFAT Reporter (Luc) – Jurkat Cell Activation by small molecule stimulators

1. Harvest NFAT Reporter – Jurkat cells from culture in Growth Medium 2B and seed cells at a density of ~ 40,000 cells per well into white clear-bottom 96-well microplate in 45 µl of assay medium.

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2. Make an intermediate dilution of activation compound (ionomycin, thapsigargin or PMA with ionomycin) by diluting T cell activating compound into assay medium at 10x desired final concentration. Add 5 µl of diluted compound to each well for a final concentration of 1x in 50 µl. Note: The final DMSO concentration can be up to 0.5%.
3. Add 5 µl of assay medium with same concentration of DMSO but without the activator to the unstimulated control wells.

4. Add 50 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).
   Note: Set up each treatment from steps 2, 3, and 4 in at least triplicate.

5. Incubate cells at 37°C in a CO_2 incubator overnight (~18 hours).

6. The next day, perform luciferase assay using the One-Step Luciferase Assay System (BPS Cat. #60690): Add 50 µl of One-Step Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.
   If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

7. 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 1. NFAT Reporter – Jurkat cell response to Thapsigargin

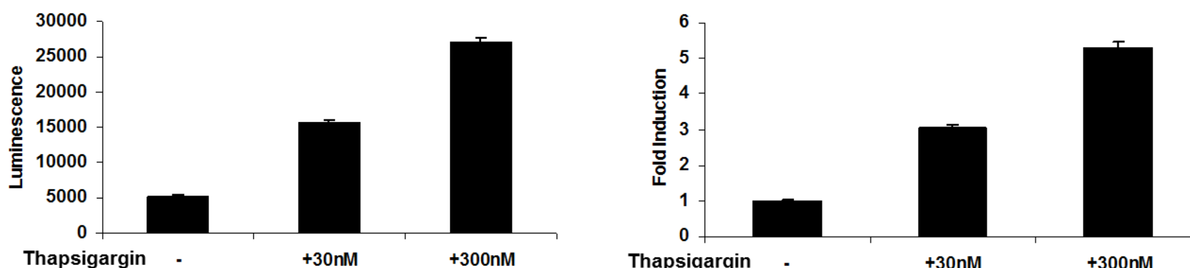

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Figure 2. NFAT Reporter – Jurkat cell response to Ionomycin.

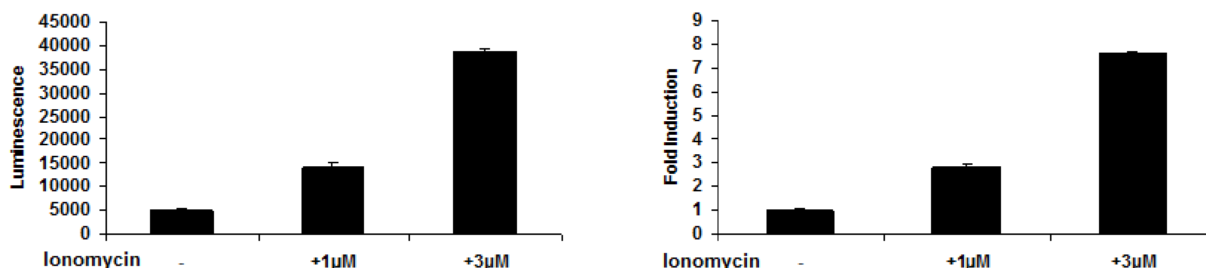
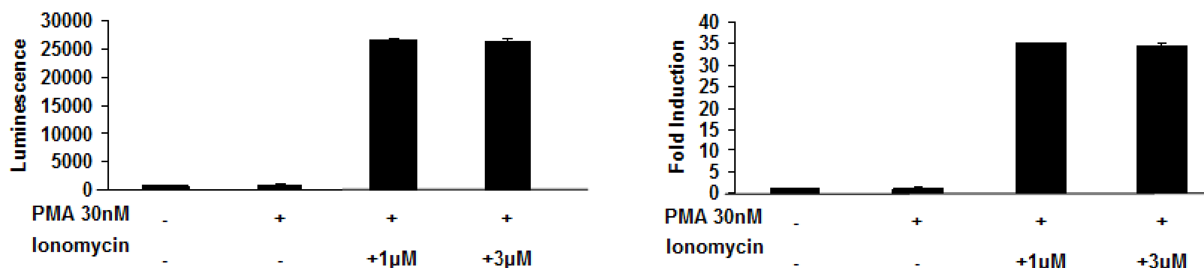


Figure 3. NFAT Reporter – Jurkat cell response to Ionomycin with 30 nM PMA.



B. NFAT Reporter (Luc) – Jurkat Cell Activation by TCR activators (TCRa/CHO, or BiTE and CAR in the presence of cancer cells)

1. Harvest TCR activator/CHO cells (BPS #60539) or CD19+ Raji cells from culture and seed cells at a density of ~30,000 cells per well into white clear-bottom 96-well microplate in 50 µl of medium, without Geneticin or Hygromycin B. *Note: it is important to optimize the CHO or Raji cell number seeded per well; the optimal number of cells can vary, based on the passage number and culture density prior to seeding*. Incubate cells at 37°C in a CO_2 incubator for 2-4 hours.
2. Harvest NFAT-reporter-Jurkat cells (log phase) by centrifugation and resuspend in assay medium. Dilute cells to 6 x 10^5 / ml in Jurkat medium. add ~30,000 cells in 50 µl of Jurkat medium per well into the above white clear-bottom 96-well microplate.
3. To test anti-CD19xanti-CD3 Bispecifc antibody (BiTE), make 10 fold serial dilution of BiTE , starting from 1nM for Blinatumomab, in Jurakt media.
4. Add 50 µl of diluted BiTE (e.g. Blinatumomab) to the above plate.
5. Add 150 µl of assay medium to cell-free control wells (for determining background luminescence).
6. Incubate at 37°C in a CO_2 incubator o/n.

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7. Next day, perform luciferase assay using the ONE-Step luciferase assay system, according to the recommended protocol. Add 150 µl of ONE-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer,.

Figure 4. NFAT Reporter – Jurkat cell response to TCRa/CHO cells

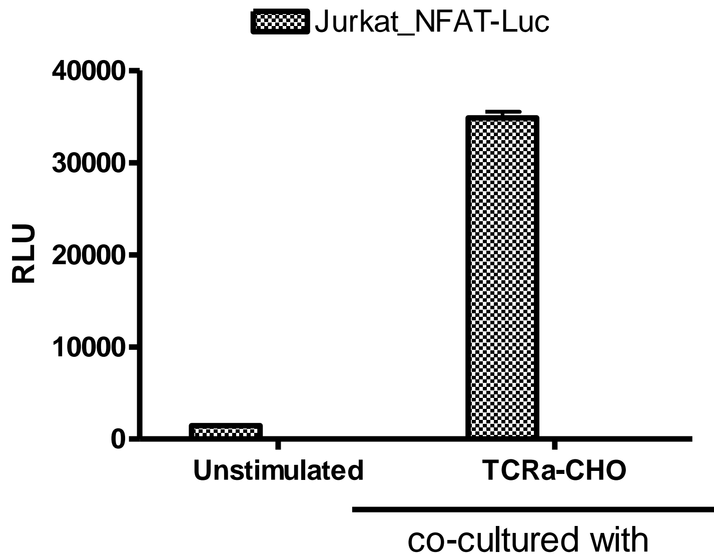

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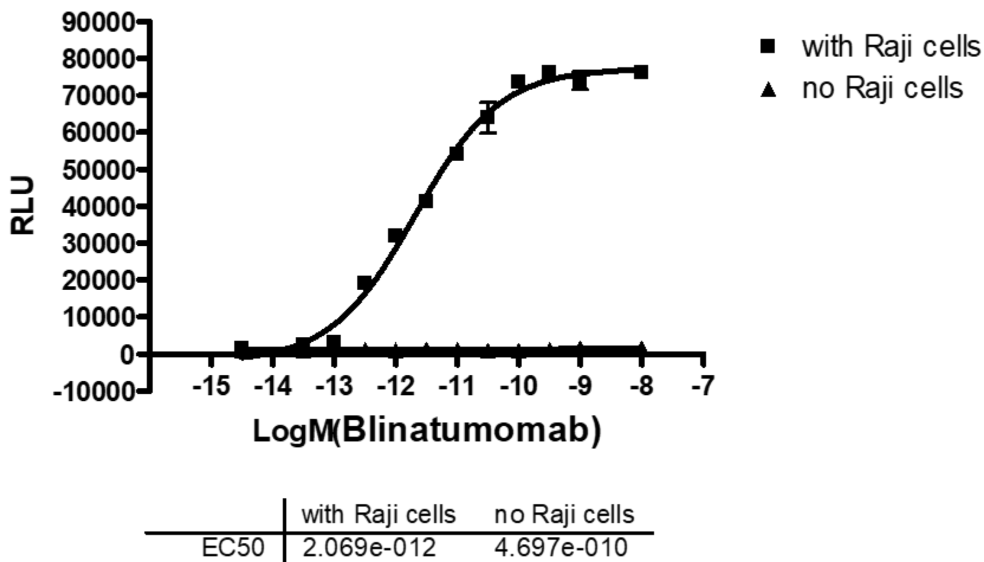
Figure 5. NFAT Reporter – Jurkat cell response to anti-CD19xanti-CD3 Bispecifc antibody (BiTE), Blinatumomab in the presence of Raji cells (CD19+)

Activation of NFAT Jurkat Reporter by CD19xCD3 BiTE in the Presence of CD19+ Raji Cells



	with Raji cells	no Raji cells
EC50	2.069e-012	4.697e-010

Figure 6. Anti-BCMA CAR NFAT reporter stable cell line activity stimulated by soluble BCMA or BCMA in CHO cells. Both soluble BCMA protein and BCMA/CHO cells caused increase of luciferase activity (4-fold with soluble BCMA and 23-fold with BCMA/CHO) by activation of NFAT through CD3ζ signaling domain downstream of anti-BCMA CAR.

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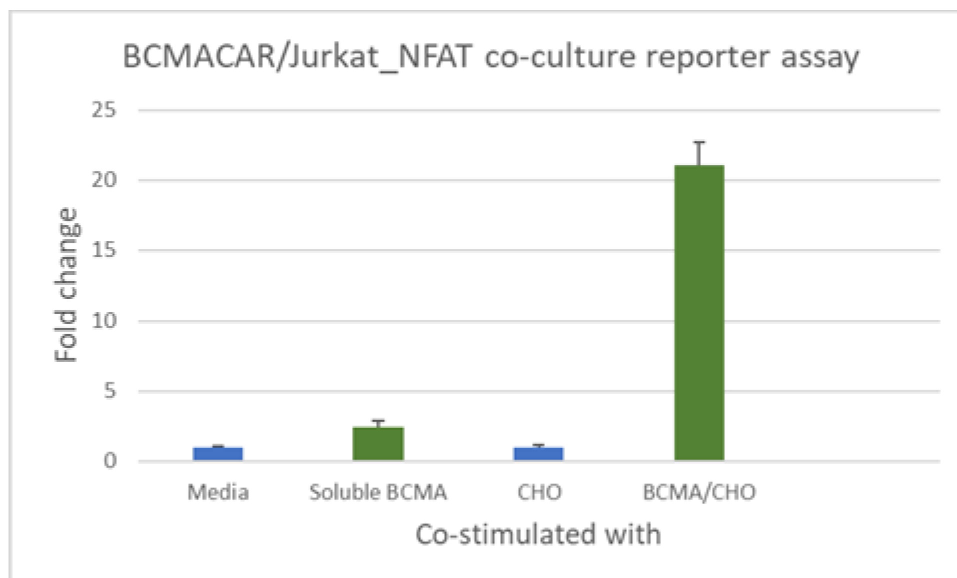

References

Clipstone NA, Crabtree GR. *Nature*. 1992 Jun 25;**357(6380):**695-7.
Lyakh, L., *et al. Mol Cell Biol.* 1997 May;**17(5):**2475-84.

Related Products

Product Name	Catalog #	Size
Anti-BCMA CAR Jurkat/NFAT (Luciferase) Reporter Cell Line	79694	2 vials
BCMA—CHO Recombinant Cell Line	79500	2 vials
PD-1 / NFAT Reporter - Jurkat Cell Line	60535	2 vials
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
ERK Signaling Pathway SRE Reporter – HEK293 Cell Line	60406	2 vials
Hedgehog Pathway Gli Reporter – NIH3T3 Cell Line	60409	2 vials
JAK/STAT Signaling Pathway ISRE Reporter – HEK293 Cell Line	60510	2 vials
JNK Signaling Pathway AP1 Reporter – HEK293 Cell Line	60405	2 vials
NK-kB Reporter (Luc) – HEK293 Cell Line	60650	2 vials
RARα Reporter (Luc) – HEK293 Cell Line	60503	2 vials
Wnt Signaling Pathway TCF/LEF Reporter – HEK293 Cell Line	60501	2 vials

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