Description

The NFAT Luciferase Reporter Jurkat Cell Line contains a firefly luciferase gene under the control of NFAT response elements stably integrated into the Jurkat cell genome. 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 PD-1.

This cell line has been functionally validated and responds to thapsigargin, ionomycin, phorbol 12-myristate 13-acetate (PMA), anti-CD3 antibodies, and Dynabeads™ Human T-Activator CD3/CD28. It can be used to measure T cell activation through a variety of TCR activators including TCR activator (anti-CD3ε scFv)/CHO cells (BPS Bioscience #60539) and CD3 x CD19 bispecific antibody (Blinatumomab) in the presence of CD19+ Raji cells.

Background

Nuclear Factor of Activated T cells (NFAT) is a family of 5 transcription factors of near ubiquitous expression, known to have a central function in the immune system, for example by inducing the expression of various cytokines (such as IL-2, IL-3, IL-4, and TNF-alpha) in T cells. NFATs cooperate with multiple other proteins to regulate distinct gene expression programs that determine the fate and function of T cell populations. The NFAT family also plays important roles in the nervous system, in the heart, and in skeletal muscles.

In resting T cells, the NFAT protein is phosphorylated and confined to the cytoplasm in an inactive state. In response to a stimulus, an influx of calcium activates the Ca2+/calmodulin-dependent serine 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 translocation to the nucleus. In the nucleus, NFAT proteins cooperate with other transcriptional regulators to induce gene expression.

Through their role in the immune system NFATs are involved in inflammation and these transcription factors are considered promising therapeutic targets for a variety of immune-related diseases.

Application

- Screen for compound activity on the PKC/ Ca2+ pathway.
- Screen for agonists or antagonists of T cell receptors.
- Control cell line for immune checkpoint NFAT reporter cell lines.
- Determine 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 Required for Cellular Assays Described in the Functional Validation section

Name	Ordering Information
Ionomycin	Sigma #I3909
Phorbol 12-myristate 13-acetate (PMA)	LC Laboratories #P1680
Thapsigargin	Sigma #T9033
Anti-CD19 x anti-CD3 bispecific antibody	BPS Bioscience #100441
Anti-CD3 antibody	BPS Bioscience #71274
Anti-CD28 antibody	BPS Bioscience #100182
Dynabeads™ Human T-Activator CD3/CD28	Thermo Fisher #11131D
GSK-7975A	Sigma #5.34351
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
White, clear-bottom cell culture plate, 96-well	
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, 1% Penicillin/Streptomycin

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

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% 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 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 NFAT Reporter (Luc) 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. The assay should be performed at least in triplicates.

Assay Medium: Thaw Medium 2 (BPS Bioscience #60184)

a. NFAT Reporter (Luc) Jurkat cell activation by small molecule agonists

1. Seed NFAT Reporter Jurkat cells in 90 µl of Thaw Medium 2 at a density of ~40,000 cells per well into white, clear-bottom 96-well plate. Keep a few wells without cells for determination of background luminescence. Each condition should be performed in triplicate.

2. Make an intermediate dilution of agonist (ionomycin, thapsigargin, or PMA with ionomycin) at a concentration 10-fold higher than the desired final concentration, in Thaw Medium 2.

   Note: The final DMSO concentration can be up to 0.5%.

   - Add 10 µl of diluted agonist to each well (final volume is 100 µl).
   - Add 10 µl of Thaw Medium 2 with the same concentration of DMSO as was used for the dilution of the agonist to measure signal in the "Unstimulated control".
   - Add 100 µl of Thaw Medium 2 to cell-free control wells (for determining background luminescence).

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

4. The next day, perform the luciferase assay using the One-Step™ Luciferase Assay System (BPS Bioscience Cat #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 NFAT luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells.

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

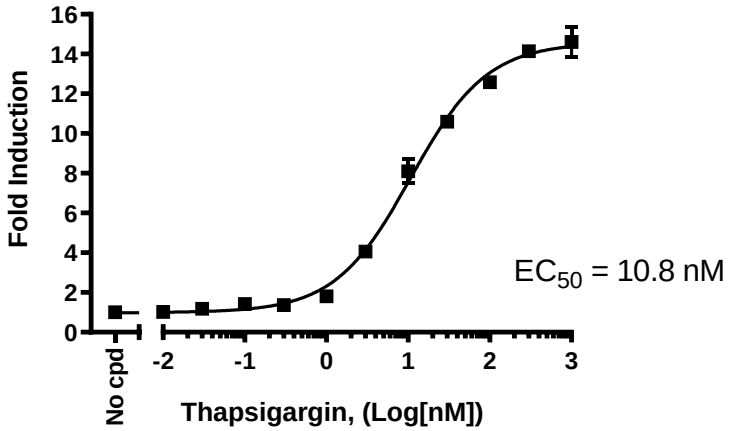

Figure 1: NFAT Reporter Jurkat cell response to Thapsigargin.

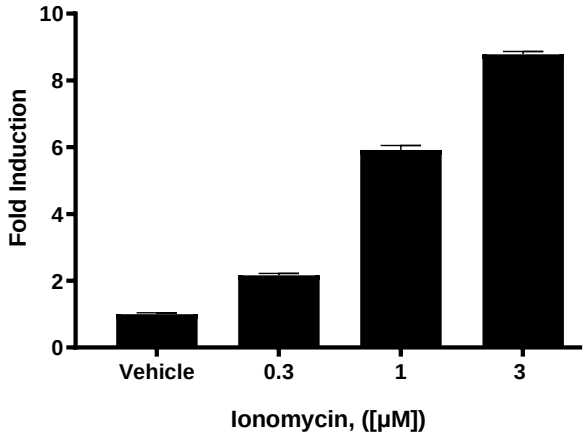

Figure 2: NFAT Reporter Jurkat cell response to Ionomycin.

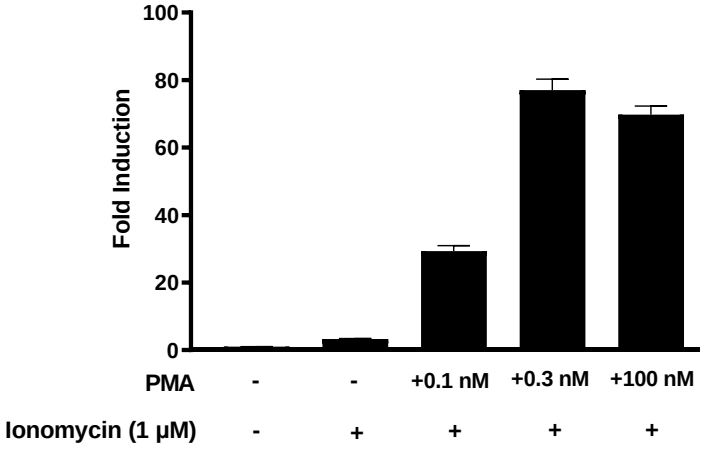

Figure 3: NFAT Reporter Jurkat cell response to PMA + Ionomycin.

b. NFAT Reporter (Luc) Jurkat cell activation in co-culture assays utilizing TCR activator CHO cells or BiTE in the presence of CD19+ Raji cells

1. Seed TCR activator CHO cells (BPS Bioscience #60539) or CD19+ Raji cells in 25 µl of Thaw Medium 2 at a density of ~30,000 cells per well into a white clear-bottom 96-well plate. Keep a few wells without cells for determination of background luminescence. Each condition should be performed in triplicate.

   Note: For co-culture models other than TCR activator CHO and Raji, it is important to optimize the number of cells per well.

2. Incubate CHO cells at 37°C in a 5% CO_2 incubator for 2-4 hours to allow attachment. Raji cells may be used immediately after plating.

3. Add the NFAT Reporter Jurkat cells in 25 µl of Thaw Medium 2 at a density of 30,000 cells per well on top of the CHO or Raji cells.

4. If testing the TCR activator/CHO cells, add an additional 50 µl Thaw Medium 2 to all wells for a final volume of 100 µl per well.

5. If testing the anti-CD19 x anti-CD3 bispecific antibody (BiTE), prepare a serial dilution of antibody at a concentration 2-fold higher than the final desired concentration in Thaw Medium 2. We recommend a dose range from 0.001 to 100 ng/ml.

   - Add 50 µl of BiTE dilutions to the treated wells containing 25 µl Raji cells and 25 µl NFAT Jurkat cells.

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

     The final incubation volume is 100 µl.

6. Incubate at 37°C in a 5% CO_2 incubator overnight.

7. The next day, perform the luciferase assay using the ONE-Step™ luciferase assay system according to the recommended protocol. Add 100 µl of ONE-Step™ Luciferase reagent per well and rock gently at room temperature for ~30 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 NFAT luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells.

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

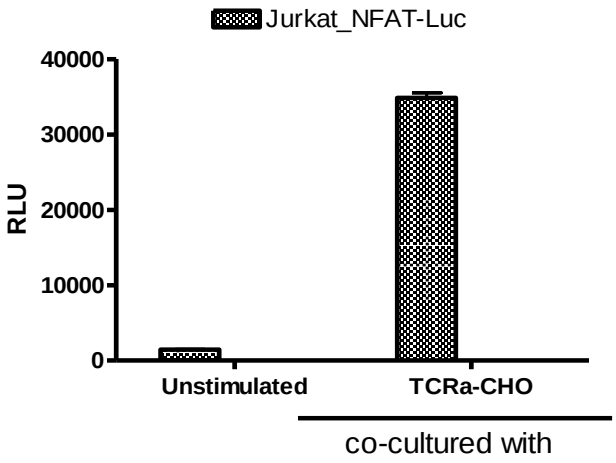

Figure 4: NFAT Reporter Jurkat cell response to TCR activator (TCRa)/CHO cells (BPS Bioscience #60539)

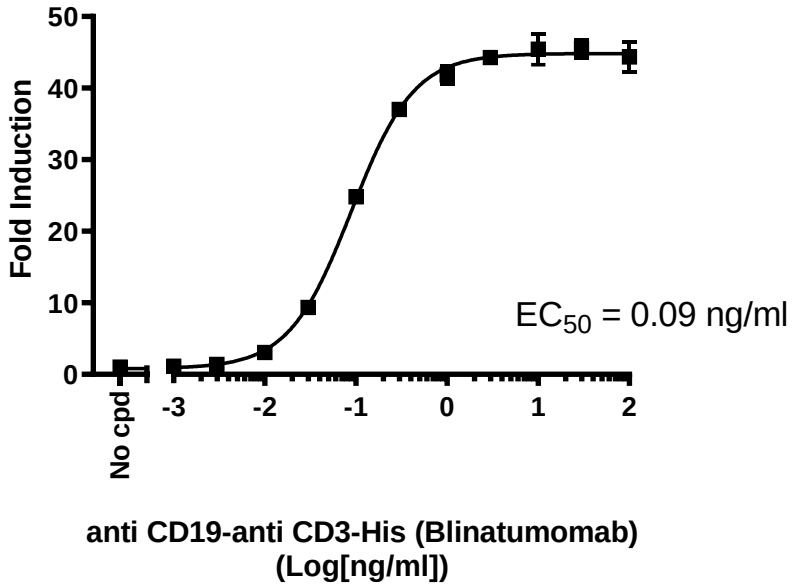

Figure 5: NFAT Reporter Jurkat cell response to anti-CD19 x anti-CD3 bispecific antibody (BPS Bioscience #100441) in the presence of CD19+ Raji cells.

c. Activation by anti-CD3 and anti-CD28 antibodies or CD3/CD28 Dynabeads™

1. Seed NFAT Luciferase Reporter Jurkat cells at a density of ~40,000 cells per well into a white clear-bottom 96-well plate in 50 µl of Thaw Medium 2. Keep a few wells without cells for determination of background luminescence. Each condition should be performed in triplicate.

2. Incubate the plate at 37°C in a 5% CO_2 incubator overnight (~18 hours).

3. The next day, prepare antibodies or CD3/CD28 Dynabeads™ (Thermo Fisher #11131D) according to protocols below:

3.1. Anti-CD3 and anti-CD28 antibodies: prepare a three-fold serial dilution of anti-CD3 antibody at concentrations 4-fold higher than the final desired concentration in Thaw Medium 2. For an EC_{50} dose curve, we recommend a dose range of approximately 0.0003 to 10 µg/ml.

- Add 25 µl of serially diluted anti-CD3 antibody to the treated wells.
- Add 50 µl of Thaw Medium 2 to internal control "No Compound" wells.
- Add 100 µl of Thaw Medium 2 to cell-free control wells (for determining background luminescence).
- Immediately after anti-CD3 addition, add 25 µl of anti-CD28 antibody at 4 µg/ml (final concentration is 1 µg/ml) to all treated wells.

The final incubation volume is 100 µl.

3.2. CD3/CD28 Dynabeads™: prepare Dynabeads™ according to the Thermo Fisher #11131D protocol. Prepare Dynabead™ dilutions in Thaw Medium 2. A 1:1 bead-to-cell ratio is recommended in the Dynabead™ protocol and is sufficient for activation of NFAT Reporter Jurkat cells.

- Add 50 µl Dynabead™ solutions to the treated wells containing 50 µl cells.
- Add 50 µl Thaw Medium 2 to internal control "cells only" wells.
- Add 100 µl of Thaw Medium 2 to cell-free control wells (for determining background luminescence).

The final incubation volume is 100 µl.

4. Incubate the plate at 37°C in a 5% CO_2 incubator for 5-6 hours.

5. 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 NFAT luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells.

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

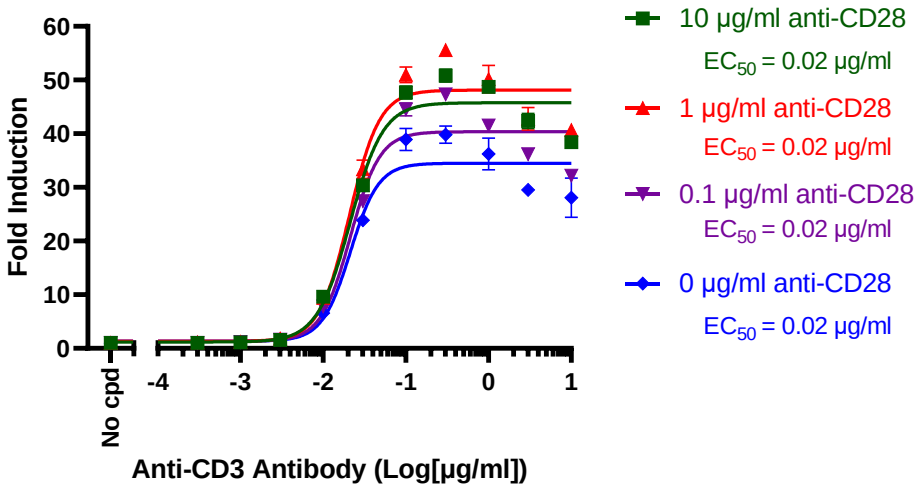

Figure 6: NFAT Reporter Jurkat cell response to anti-CD3 and anti-CD28 antibodies.
Cells were co-stimulated by increasing concentrations of anti-CD3 antibody (clone OKT3, BPS Bioscience #71274) in the presence of a fixed concentration of anti-CD28 antibody (BPS Bioscience #100182).

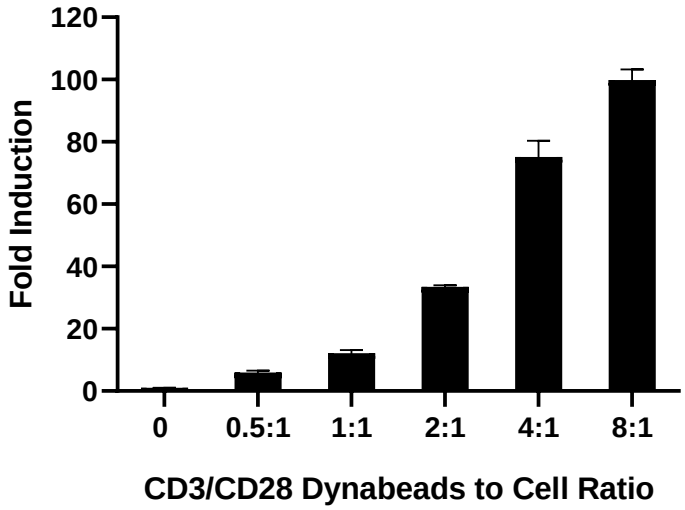

Figure 7: NFAT Reporter Jurkat cell response to CD3/CD28 Dynabeads™.
Cells were incubated with the indicated bead-to-cell ratios of Human T-Activator CD3/CD28 Dynabeads™ (Thermo Fisher #11131D). Dynabeads are a trademark of Thermo Fisher Scientific.

d. Inhibition of PMA/Ionomycin-induced NFAT activity by CRAC Inhibitor GSK-7975A

1. Seed NFAT Luciferase Reporter Jurkat cells at a density of ~40,000 cells per well into a white clear-bottom 96-well plate in 50 µl of Thaw Medium 2. Keep a few wells without cells for determination of background luminescence. Each condition should be performed in triplicate.

2. Prepare a serial dilution of inhibitor at concentrations 4-fold higher than the final desired concentration in Thaw Medium 2. As shown in our example below, we performed a dose response of GSK-7975A using a final dose range of 0.0003 to 10 μM.

    - Add 25 μl of serially diluted inhibitor to the treated wells.
    - Add 25 μl of Thaw Medium 2 to control "No Inhibitor" wells.
    - Add 25 μl of Thaw Medium 2 to internal control "No Inhibitor and No Agonist" wells.
    - Add 100 μl of Thaw Medium 2 to cell-free control wells (for determining background luminescence).

3. Incubate the plate at 37°C in a 5% CO_2 incubator for 30 minutes.

4. Prepare a solution of PMA + ionomycin at concentrations 4-fold higher than the final desired concentrations: [PMA] = 15 nM and [Ionomycin] = 0.5 μM.

    - Add 25 μl of the PMA and ionomycin mix to the "Test Inhibitor" wells and to the control "No Inhibitor" wells.
    - Add 25 μl of Thaw Medium 2 to internal control "No Inhibitor and No Agonist" wells.

5. Incubate the plate at 37°C in a 5% CO_2 incubator overnight.

6. 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.

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

$$Percent\ luminescence = \left(\frac{luminescence\ of\ \ treated\ wells - avg.background}{avg.luminescence\ of\ untreated\ wells - avg.background}\right) x100$$

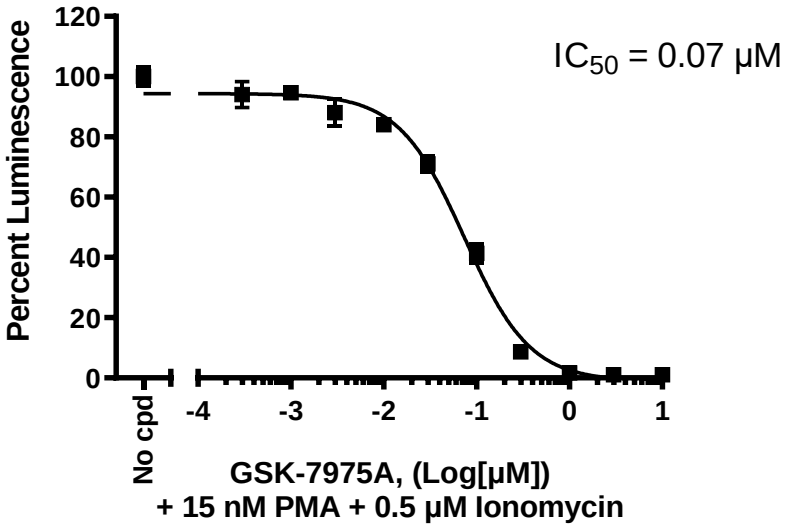

Figure 8: NFAT Reporter Jurkat cell response to GSK-7975A, an inhibitor of calcium release activated channels.
Cells were treated with increasing concentrations of inhibitor for 30 minutes before addition of agonists PMA [15 nM] and ionomycin [0.5 µM].

References

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

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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*
Anti-BCMA CAR Jurkat/NFAT (Luciferase) Reporter Cell Line	79694	2 vials
PD-1 / NFAT Reporter - Jurkat Cell Line	79500	2 vials
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
JAK/STAT Signaling Pathway ISRE Reporter – HEK293 Cell Line	60510	2 vials
TIGIT / NFAT Reporter Jurkat Cell Line	60538	2 vials
Firefly Luciferase Jurkat Cell Line	78373	2 vials