

Data Sheet

NFAT Reporter (Luc) – Jurkat Cell line

Catalog #: AMS.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-CD3 ϵ scFv)/CHO cells (AMS.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/ Ca²⁺ 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)
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Format

Each vial contains ~2x10⁶ cells in 1 ml of 10% DMSO.

Storage

Immediately upon receipt, store in liquid nitrogen.

Culture conditions

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

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

Cells should be grown at 37°C with 5% CO₂ 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% CO₂ incubator. At first passage, switch to Growth Medium 2B (**contains geneticin**). Cells should be split before they reach 2x10⁶ cells/ml. To passage the cells, dilute cell suspension into new culture vessels at no less than 0.1 x10⁶ 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 (Cat. AMS.60184)
- Growth Medium 2B (Cat. AMS.79530)
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- One-Step™ luciferase assay system (AMS.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 μ l of diluted compound to each well for a final concentration of 1x in 50 μ l. Note: The final DMSO concentration can be up to 0.5%.
3. Add 5 μ l of assay medium with same concentration of DMSO but without the activator to the unstimulated control wells.
4. Add 50 μ l 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 CO₂ incubator overnight (~18 hours).
6. The next day, perform luciferase assay using the One-Step Luciferase Assay System (Cat. #60690): Add 50 μ l 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

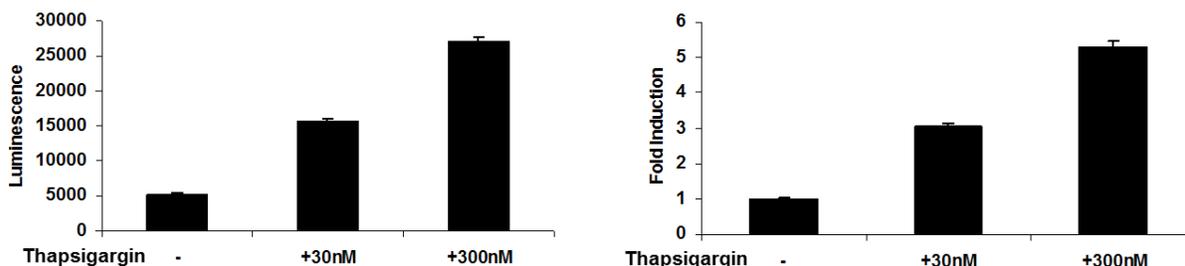


Figure 2. NFAT Reporter – Jurkat cell response to Ionomycin.

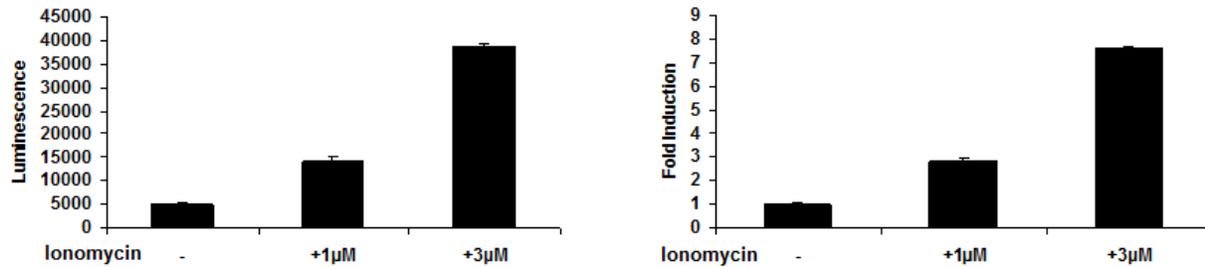
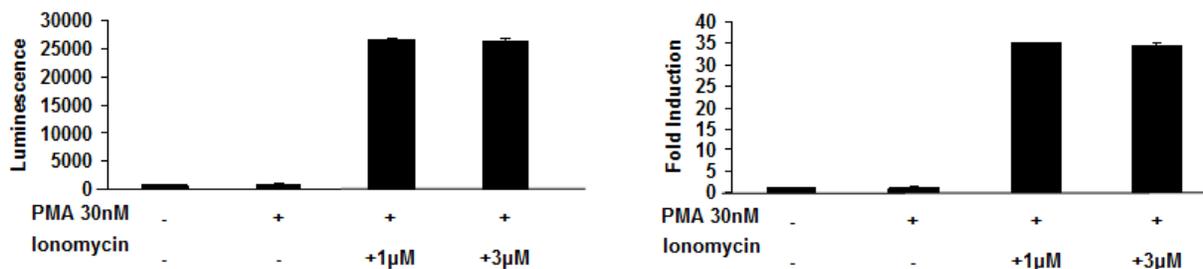


Figure 3. NFAT Reporter – Jurkat cell response to Ionomycin with 30 nM PMA.



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 (AMS.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 CO₂ 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 10⁵ / 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 Bispecific antibody (BiTE), make 10 fold serial dilution of BiTE, starting from 1nM for Blinatumomab, in Jurkat 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 CO₂ incubator o/n.

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- Next day, perform luciferase assay using the ONE-Step luciferase assay system, according to the recommended protocol. Add 150 μ l 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

**Jurkat_NFAT Reporter Cells Activation by TCRa/CHO
Co-culture Luciferase Assay
(n=8)**

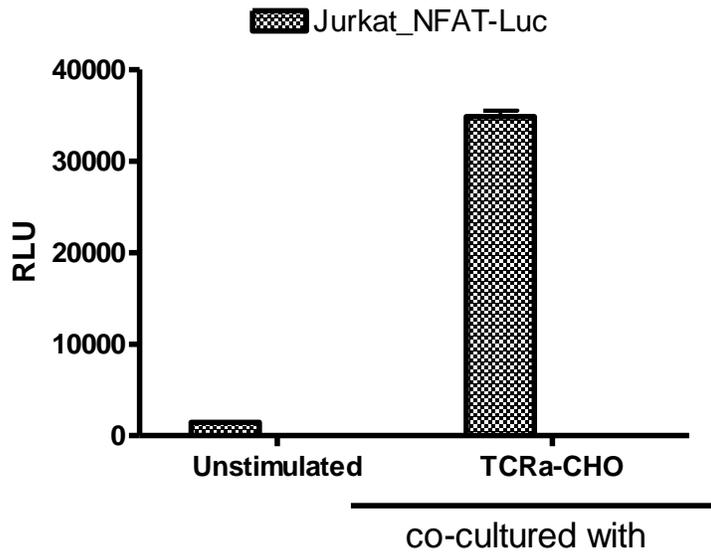
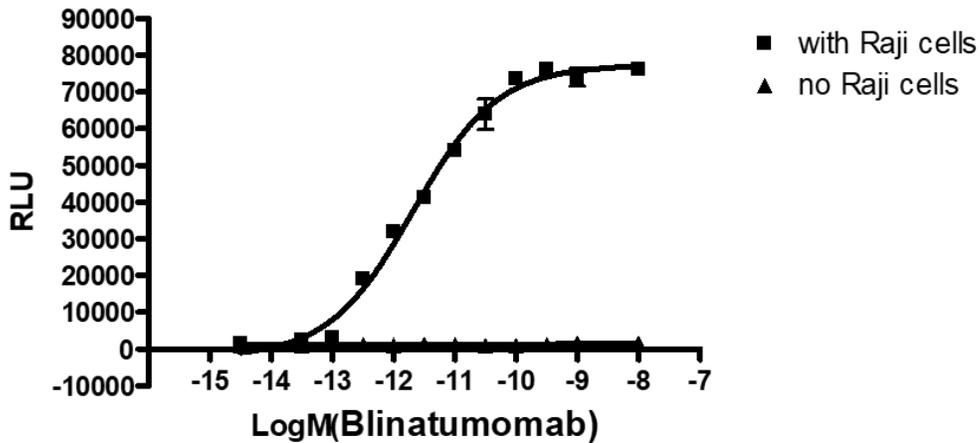


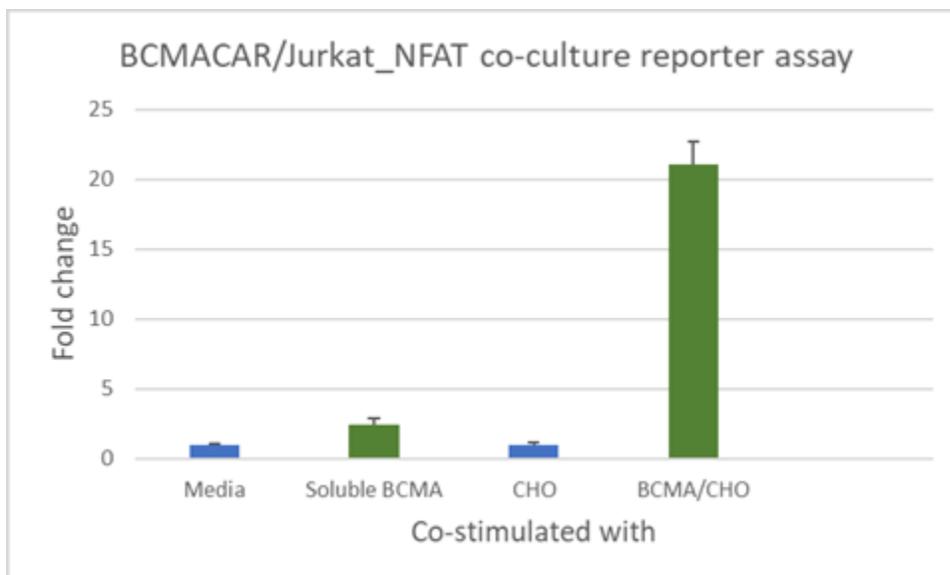
Figure 5. NFAT Reporter – Jurkat cell response to anti-CD19xanti-CD3 Bispecific antibody (BiTE), Blinatumomab in the presence of Raji cells (CD19+)

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 CD3 ζ signaling domain downstream of anti-BCMA CAR.



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

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

License Disclosure

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell,

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