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Data Sheet

NFAT Reporter – HEK293 Cell line
PKC/ Ca^{2+} Pathway
Catalog #: 79298

Background
The protein kinase c (PKC)/ Ca^{2+} response pathway leads to activation of the transcription factor nuclear factor of activator T cells (NFAT). NFAT is regulated by Ca2+ and the Ca2+/calmodulin-dependent serine phosphatase calcineurin. NFAT proteins are phosphorylated and reside in the cytoplasm in resting cells; upon stimulation, they are dephosphorylated by calcineurin, translocate to the nucleus, and induce gene expression.

Product Description
The NFAT Reporter – HEK293 cell line contains a firefly luciferase gene under the control of NFAT response element stably integrated into HEK293 cells. This cell line is validated for the response to the stimulation of phorbol 12-myristate 13-acetate (PMA) with ionomycin.

Application
- Monitor intracellular calcium levels.
- Screen for activators or inhibitors of the PKC/ Ca^{2+} pathway.

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

Storage
Immediately upon receipt, store in liquid nitrogen.

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.

Mycoplasma testing
The cell line has been screened using the PCR-based VenorGeM® Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of Mycoplasma species.

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Culture Conditions:

Thaw Medium 1 (BPS Bioscience, #60187): Optimized cell culture medium for thawing and plating HEK293 cells. Includes 10% FBS, non-essential amino acids, sodium pyruvate and 1% Penicillin/Streptomycin.

Growth Medium 1B (BPS Cat. #79531): Thaw Medium 1 (BPS Bioscience, #60187) with 400 µg/ml of Geneticin (Life Technologies #11811031).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1B. It may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1, spin down cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 1. 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 1B. Cells should be split before they reach 100% confluence. To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA, add Growth Medium 1B and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~ 1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with 1:8 -1:20 ratio weekly.

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.
- Assay medium: Thaw Medium 1 (no Geneticin)
- Growth Medium 1B (BPS Cat. #79531)
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- RO-31-8220 (EMD Millipore, #557520) or other PKC inhibitor
- ONE-Step Luciferase Detection Reagents (BPS Bioscience, #60690) for measuring firefly luciferase activity.
- Luminometer

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A. Response of NFAT Reporter – HEK293 cells to TCR crosslinkers.

1. Harvest NFAT Reporter – HEK293 cells from culture in Growth Medium 1B and seed cells at a density of ~ 30,000 cells per well into white clear-bottom 96-well microplate in 45 µl of assay medium.

2. Incubate the plate at 37°C in a CO_2 incubator overnight (~18-24 hours).

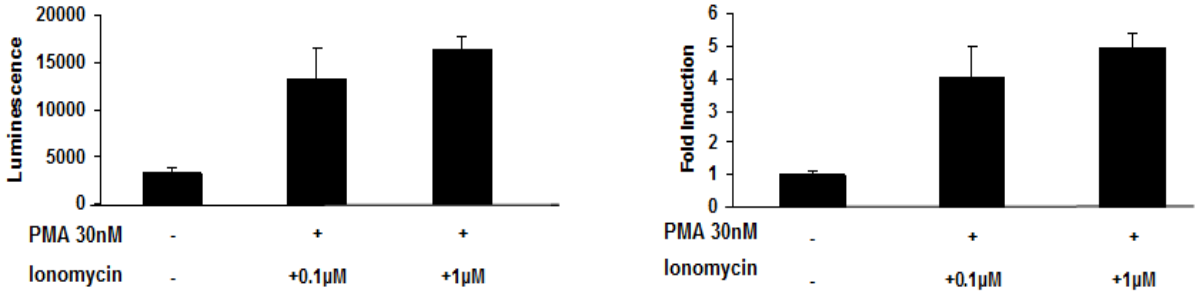
3. Dilute TCR crosslinker (PMA with ionomycin) into assay medium at 10x desired final concentration and add 5 µl of dilution to each well. (We recommend a starting concentration around 30 nM PMA and 1 µM Ionomycin.) The final DMSO concentration can be up to 0.5%.
   Add 5 µl of assay medium with same concentration of DMSO but without the crosslinker to the unstimulated control wells.
   Add 50 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

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

5. The next day, perform luciferase assay using the ONE-Step luciferase assay system: Add 50 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 = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 1. NFAT Reporter (Luc) – HEK293 cell response to TCR Crosslinker Ionomycin with PMA.



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B. Response of NFAT Reporter – HEK293 cells to Inhibitors of TCR crosslinkers.

1. Harvest NFAT Reporter – HEK293 cells from culture in Growth Medium 1B and seed cells at a density of ~ 30,000 cells per well into white clear-bottom 96-well microplate in 45 µl of assay medium.

2. Incubate the plate at 37°C in a CO_2 incubator overnight (~18-24 hours).

3. Dilute inhibitor (RO-31-8220) into assay medium to 10x desired final concentration and add 5 µl of dilution to each well. The final DMSO concentration can be up to 0.5%.
   Add 5 µl of assay medium with same concentration of DMSO but without the inhibitor to the un-inhibited control wells.

4. Dilute TCR crosslinker (PMA with ionomycin) into assay medium to 10x desired final concentration (We recommend a starting concentration around 30 nM PMA and 1 µM Ionomycin) and add 5 µl of dilution to each well. The final DMSO concentration can be up to 0.5%.
   Add 5 µl of assay medium with same concentration of DMSO but without the crosslinker to the unstimulated control wells.
   Add 55 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

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

   The next day, perform luciferase assay using the ONE-Step luciferase assay system: Add 55 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 percent luminescence of NFAT luciferase reporter expression is calculated by dividing each condition by the background-subtracted luminescence of the stimulated well. The background-subtracted luminescence of cells stimulated with 1 µM Ionomycin and 30 nM PMA in the absence of RO-31-8220 was set at 100%.

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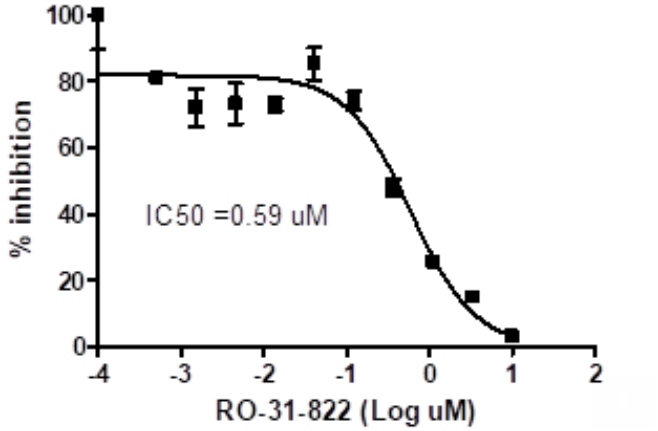
Figure 2. Inhibition of PKC by RO-31-8220 in PMA and Ionomycin induced NFAT reporter (Luc)-HEK293 cells.
The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with 1 µM Ionomycin and 30 nM PMA in the absence of RO-31-8220 was set at 100%.



Related Products

Product Name	Catalog #	Size
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	60621	2 vials
TIGIT / NFAT Reporter - Jurkat Cell Line	60538	2 vials
PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line	60535	2 vials
LAG3 / NFAT Reporter - Jurkat Recombinant Cell Line	71278	2 vials
PKCα (PKCalpha), GST-tag	40157	10 µg
PKCβ I (PKCbeta1), GST-tag	40158	10 µg
PKCβ II (PKCbeta1), GST-tag	40159	10 µg
PKCγ (PKCgamma), GST-tag	40160	10 µg
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
RARalpha Reporter (Luc) – HEK293 Cell Line	60503	2 vials
Wnt Signaling Pathway TCF/LEF Reporter – HEK293 Cell Line	60501	2 vials

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