BOSTER BIOLOGICAL TECHNOLOGY
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NFAT Luciferase Reporter-RAW264.7 Cell Line

Catalog number: RC1011

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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Catalog Number: RC1011, **Storage:** Immediately upon receipt, store in liquid nitrogen. (Ship on dry ice.)

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Contents: Each vial contains 2 ~ 3 x 10^6 cells in 1 ml of 90% FBS + 10% DMSO.
Description: The NFAT Luciferase Reporter cell line is a stably transfected RAW264.7 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the Nuclear Factor of Activated T-cells (NFAT) response element, so that the cell line is designed to measure the transcriptional activity of NFAT. NFAT is a transcription factor originally found in activated T lymphocytes, and is now known to regulate not only T cell activation and differentiation but also the function of other immune cells including dendritic cells, B cells and megakaryocytes. The NFAT induction by calcium ionophore A23187 is shown in Figure 1.
Applications: Functional Assay
Application Notes: Functional Assay, detecting the transcriptional activity of NFAT
Application Details:

Application:

Monitor the NFAT signaling pathway activity.Screen for activators or inhibitors of the NFAT signaling pathway.

Culture conditions:

Cells should be grown at 37°C with 5% CO2 using DMEM medium supplemented with 10% FBS and 1% Pen/Strep, plus 3 μg/ml of Puromycin.It is recommended to quickly thaw the frozen cells upon receipt or from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of growth medium without Puromycin, spin down cells, resuspend cells in pre-warmed growth medium without Puromycin, transfer resuspended cells to T25 flask and culture in 37°C-CO2 incubator. Leave the T25 flask in the incubator for 1~2 days without disturbing or changing the medium until cells completely recover viability and become adherent. Once cells are over 90% adherent, remove growth medium and passage the cells through trypsinization and centrifugation. At first passage, switch to growth medium containing Puromycin. Cells should be split before they reach complete confluence.To passage the cells, detach cells from culture vessel with Trypsin/EDTA, add complete growth medium and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cells suspension into new culture vessels. Subcultivation ration = 1:10 to 1:20 weekly.

Functional validation:

A. Response of NFAT RAW264.7 cells to calcium ionophore A23187. 1. Harvest NFAT RAW264.7 cells and seed cells into a white solid-bottom 96-well microplate in 100 μl of growth medium at 8.5 x 10^4 cells/well. 2. Incubate cells at 37°C in a CO2 incubator for overnight. 3. The next day, stimulate cells with different concentrations of calcium ionophore A23187. 4. Incubate at 37°C in a CO2 incubator for 6-16 hours. 5. Add 50 μl of luciferase assay reagent per well. 6. Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer.

NFAT Luciferase Reporter-RAW264.7 Cell Line (RC1011) Images

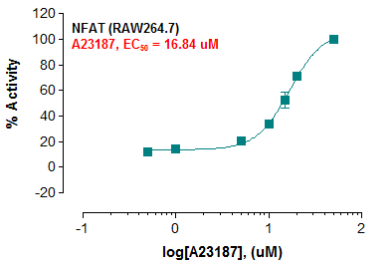

Fig-1: Induction of NFAT activity by calcium ionophore A23187 in NFAT RAW264.7 cells.

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