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# 14-111ACL: NFAT Reporter – RAW264.7 Cell Line

**Application :** Functional Assay

## Description

The NFAT 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.

## Product Info

| | |
|---|---|
| **Amount :** | 1 Vial |
| **Content :** | Each vial contains 2 ~ 3 x 10^6 cells in 1 ml of 90% FBS + 10% DMSO. |
| **Storage condition :** | Immediately upon receipt, store in liquid nitrogen. |

## Application Note

**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% $CO_2$ using DMEM medium (w/ L-Glutamine, 4.5g/L Glucose and Sodium Pyruvate) supplemented with 10% heat-inactivated FBS and 1% Pen/Strep, plus 3 µg/ml of Puromycin (Note: Puromycin can be omitted during the reporter cell assays).

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-$CO_2$ 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. ***Note: RAW264.7 cells may not be detached well by trypsinization only. So you may need to use a cell scraper to harvest the trypsinized cells.***

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. To achieve satisfactory results, cells should not be passaged over 16 times.

**Functional validation:**

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A. Response of NFAT Leeporter™ – RAW264.7 cells to calcium ionophore A23187.

1. Plate NFAT Leeporter™ – RAW264.7 cells into a white solid-bottom 96-well microplate in 100 ul of growth medium at 1 x $10^5$ cells/well and incubate cells at 37°C in a $CO_2$ incubator for 4-6 hours.

2. Stimulate cells with different concentrations of calcium ionophore A23187 and incubate cells at 37°C in a $CO_2$ incubator for 16 hours. ,

3. Equilibrate the plate to room temperature for 10 minutes.

4. Add 50 µl of luciferase assay reagent (Abeomics, Cat #17-1101; Refer to the reagent datasheet for the detailed luciferase assay protocol) per well.

5. Read the plate in 1-5 minutes to measure luminescence using a microplate luminometer.

**LIMITED USE RESTRICTIONS:**

**THIS PRODUCT IS SOLELY FOR IN VITRO RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.**

**By use of this product, user agrees to be bound by the terms of this limited use statement.**

**This product is solely for Internal Research Purposes and not for Commercial Purposes. Commercial Purposes include, but are not limited to (1) use of the cell line in manufacturing; (2) use of the cell line to provide a service, information or data; (3) use of the cell line for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the cell line whether or not such cell lines are resold for use in research. The buyer cannot sell, give or otherwise transfer this product to a third party.**

**Commercial License Agreement is available for non-research use if applicable. Please contact Abeomics (info@abeomics.com).**

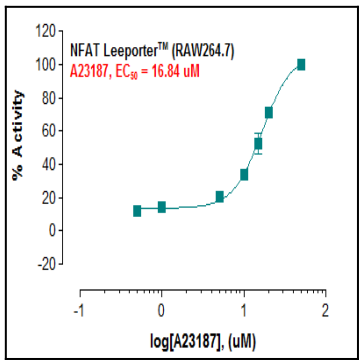


Fig-1: Induction of NFAT activity by calcium ionophore A23187 in NFAT Leeporter™ – RAW264.7 cells.