

## 14-110ACL: NFAT LEEPORTER™ Luciferase Reporter-HEK293 Cell Line

**Application :** Functional Assay

### Description

The NFAT LEEPORTER™ Luciferase Reporter cell line is a stably transfected HEK 293 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

<b>Amount :</b>	1 Vial
<b>Content :</b>	Each vial contains $2 \sim 3 \times 10^6$ cells in 1 ml of 90% FBS + 10% DMSO.
<b>Storage condition :</b>	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<sub>2</sub> 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<sub>2</sub> incubator.

Leave the T25 flask in the incubator for 1~3 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. To achieve satisfactory results, cells should not be passaged over 16 times.

## Functional validation:

### A. Response of NFAT LEEporter™ – HEK293 cells to calcium ionophore A23187.

1. Harvest NFAT LEEporter™ – HEK293 cells and seed cells into a white solid-bottom 96-well microplate in 100  $\mu$ l of growth medium at  $5 \times 10^4$  cells/well.
2. Incubate cells at 37°C in a CO<sub>2</sub> incubator for overnight.
3. The next day, stimulate cells with different concentrations of calcium ionophore A23187.
4. Incubate at 37°C in a CO<sub>2</sub> incubator for 16 hours.
5. Equilibrate the plate to room temperature for 10 minutes.
6. Add 50  $\mu$ l of luciferase assay reagent (Abeomics, Cat #17-1101; Refer to the reagent datasheet for the detailed luciferase assay protocol) per well.
7. Read the plate in 1-5 minutes to measure luminescence using a microplate luminometer.

## LIMITED USE RESTRICTIONS:

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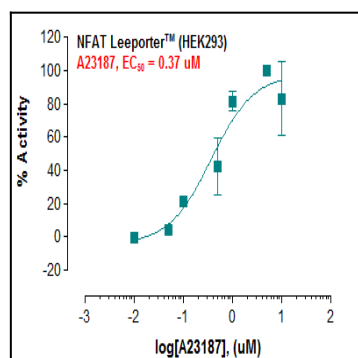


Fig-1: Induction of NFAT activity by calcium ionophore A23187 in NFAT LEEporter™ - HEK293 cells.