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14-150ACL: NFAT Leeporter™ Luciferase Reporter-Jurkat Cell Line

Application : Functional Assay

Description

The NFAT Leeporter™ Luciferase Reporter cell line is a stably transfected Jurkat T 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 anti-CD3 antibody and ionomycin is shown in Figures 1 through 4.

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 RPMI medium supplemented with 10% heat-inactivated FBS, 1 mM sodium pyruvate, 10 mM HEPES 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.

Monitor the cell viability by counting cells daily for 1~3 days until cells completely recover viability as cells are doubling daily. Once cells are over 90% confluent, harvest cells by centrifugation and passage cells. At first passage, switch to growth medium containing Puromycin. Cells should be split before they reach complete confluence.

To passage the cells, transfer cells to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell 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™ – Jurkat cells to anti-CD3 antibody.

1. Harvest NFAT Leeporter™ – Jurkat cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 2.5 x 10^5 cells/well.

2. Right after plating cells, stimulate cells with various concentrations of anti-CD3 antibody in the presence or absence of anti-CD28 antibody (at 10 ug/ml), 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.

B. Response of NFAT Leeporter™ – Jurkat cells to Ionomycin.

1. Harvest NFAT Leeporter™ – Jurkat cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 2.5 x 10^5 cells/well.

2. Right after plating cells, stimulate cells with various concentrations of ionomycin in the presence or absence of PMA (at 10 ng/ml), 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.

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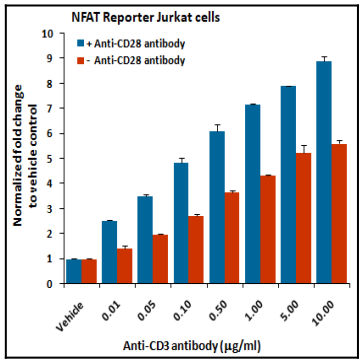

Fig-1: Induction of NFAT activity by anti-CD3 antibody in the presence or absence of anti-CD28 antibody at 10 ug/ml in NFAT Leeporter™ - Jurkat T cells.

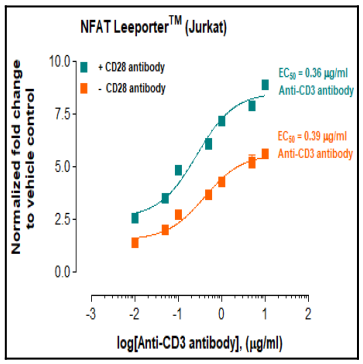

Fig-2: EC50 evaluation of anti-CD3 antibody against NFAT induction in NFAT Leeporter™ - Jurkat T cells.

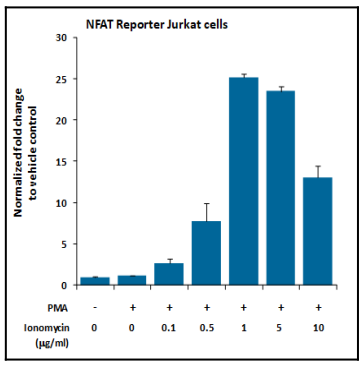

Fig-3: Induction of NFAT activity by Ionomycin in the presence of PMA in NFAT Leeporter™ - Jurkat T cells.

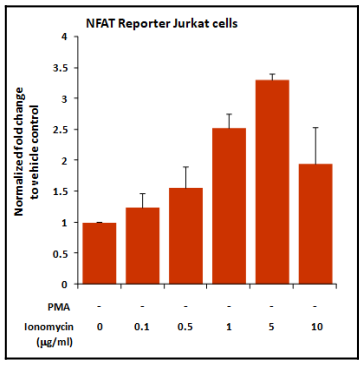

Fig-4: Induction of NFAT activity by Ionomycin in the absence of PMA in NFAT Leeporter™ - Jurkat T cells.