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14-142ACL: NF-kB Leeporter™ Luciferase Reporter-Jurkat Cell Line

Application : Functional Assay

Description

The NF-kB 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 NF-kB response element. NF-kB is a key transcription factor that is involved in immune and inflammatory responses, developmental processes, cellular growth and apoptosis. The NF-kB induction by phorbol 12-myristate 13-acetate (PMA) 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 NF-kB signaling pathway activity.
- Screen for activators or inhibitors of the NF-kB 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:

A. Response of NF-kB Leeporter™ – Jurkat cells to phorbol 12-myristate 13-acetate (PMA).

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1. Harvest NF-kB 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 PMA 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 NF-kB Leeporter™ – Jurkat cells to anti-CD3 antibody.

1. Harvest NF-kB 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.

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.

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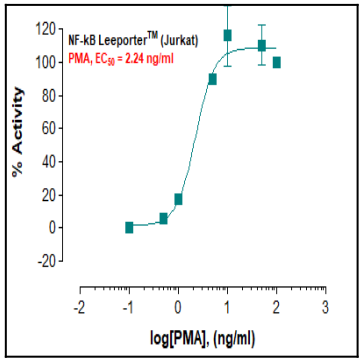


Fig-1: Induction of NF-kB activity by PMA in NF-kB Leeporter™ - Jurkat T cells.

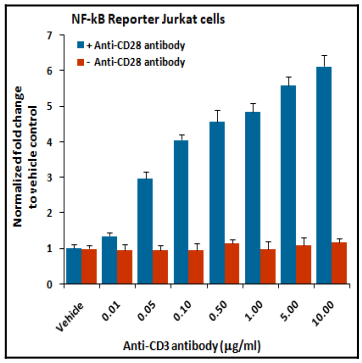


Fig-2: Induction of NF-kB activity by anti-CD3 antibody in the presence or absence of anti-CD28 antibody (at 10 ug/ml) in NF-kB Leeporter™ - Jurkat T cells.

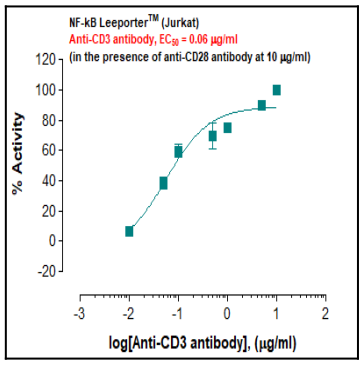


Fig-3: EC50 evaluation of anti-CD3 antibody against NF-kB induction in NF-kB Leeporter™ - Jurkat T cells.

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