9853 Pacific Heights Blvd.
Suite D. San Diego,
CA 92121, USA
Tel: 858-263-4982
Email: info@abeomics.com

14-144ACL: FOXP3 Leeporter™ Luciferase Reporter-Jurkat Cell Line

Application : Functional Assay

Description

The FOXP3 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 Forkhead box P3 (FOXP3) promoter. As a member of the forkhead transcription factor family, FOXP3 is a key transcription factor that functions in the development and function of regulatory T cells. Functional activity of the cell line has been validated by phorbol 12-myristate 13-acetate (PMA) in the presence of ionomycin (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 FOXP3 induction activity.
- Screen for activators or inhibitors of FOXP3 indction.

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 FOXP3 Leeporter™ – Jurkat T cells to phorbol 12-myristate 13-acetate (PMA)/ Ionomycin.

1. Harvest FOXP3 Leeporter™ – Jurkat T 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 in the presence of 0.2 uM ionomycin and incubate cells at 37°C in a CO_2 incubator for up to 24 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.

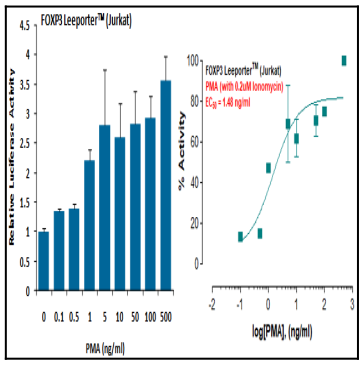

Fig-1: Induction of NF-kB activity by PMA in FOXP3 Leeporter™ - Jurkat T cells.