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14-151ACL: CD27/NF-kB Leeporter™ Luciferase Reporter-Jurkat Cell Line

Application : Functional Assay

Description

The CD27/NF-kB Leeporter™ Luciferase Reporter cell line is a stably transfected Jurkat cell line which expresses full-length human CD27 along with the Renilla luciferase reporter gene under the transcriptional control of the NF-kB response element. CD27 is a tumor necrosis factor (TNF) receptor superfamily member found on T cells, B cells and NK cells. It binds to its ligand, CD70, which is present on activated lymphocytes and dendritic cells, triggering a co-stimulatory signal that facilitates T cell activation, survival and proliferation. Targeting the CD27/CD70 pathway has emerged as a promising therapeutic approach for treating both innate and adaptive immune disorders, as well as cancer.

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 T cell co-stimulatory signaling pathway activity.
- Screen for activators or inhibitors of the CD27/CD70 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 and 10 µg/ml Blasticidin (Note: Puromycin and Blasticidin 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 and Blasticidin, 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 and Blasticidin. 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.

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Functional validation:

A. Activation of CD27 signaling pathway via co-stimulation with CD70 stably expressed in the CD70/CHO-K1 stable cell line.

1. Harvest CD70/CHO-K1 cells (Abeomics, Cat. No. 14-543ACL) and seed cells into a white solid-bottom 96-well microplate in 100 µl of DMEM growth medium at different number of cells (i.e. 2-fold serial dilutions starting from 1 x 10^5 cells/well).

2. Next day, remove the medium, and add CD27/NF-kB Leeporter™ – Jurkat cells in 100 µl of RPMI growth medium at 2 x 10^4 cells/well.

3. Incubate cells at 37°C in a CO_2 incubator for 6 hours.

4. After 6 hours, equilibrate the plate to room temperature for 10 minutes.

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

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

B. Activation of CD27 signaling pathway via co-stimulation with CD70 naturally expressed in Raji cells.

1. Harvest Raji cells and seed cells into a white solid-bottom 96-well microplate in 50 µl of RPMI growth medium at different number of cells (i.e. 2-fold serial dilutions starting from 1 x 10^5 cells/well).

2. Harvest CD27/NF-kB Leeporter™ – Jurkat cells and add cells to the plate containing Raji cells in 50 µl of RPMI growth medium at 2 x 10^4 cells/well.

3. Incubate cells at 37°C in a CO_2 incubator for 6 hours.

4. After 6 hours, equilibrate the plate to room temperature for 10 minutes.

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

6. 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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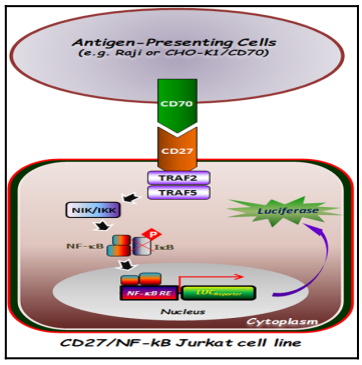

Fig-1: CD27/CD70 signaling pathway. CD27, a TNF receptor superfamily member is activated by its unique ligand CD70, leading to activation of innate and adaptive immunity. CD27 in the Jurkat/NF-kB/hCD27 Reporter Cell Line can be activated by CD70 stably expressed in the hCD70/CHO-K1 Cell Line (Cat. No. 14-543ACL) or naturally expressed in Raji cells.

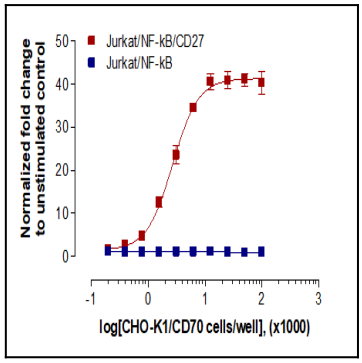

Fig-2: Activation of CD27 signaling pathway. CD27 in the Jurkat/NF-kB/hCD27 Reporter Cell Line was activated by CD70 stably expressed in hCD70/CHO-K1 Cell Line (Cat. No. 14-543ACL) in a dose-response manner. Note: Jurkat/NF-kB reporter cell line (Abeomics, Cat. No. 14-142ACL) was used as control.

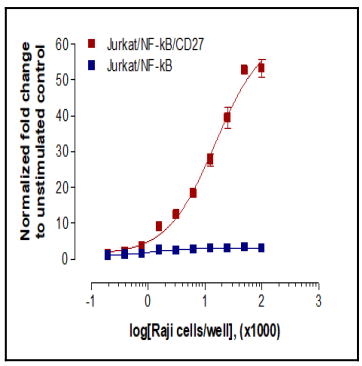

Fig-3: Activation of CD27 signaling pathway. CD27 in the Jurkat/NF-kB/hCD27 Reporter Cell Line was activated by CD70 naturally expressed in Raji cells in a dose-response manner. Note: Jurkat/NF-kB reporter cell line (Abeomics, Cat. No. 14-142ACL) was used as control.