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14-100ACL: NF-kB Leeporter™ Luciferase Reporter-RAW264.7 Cell Line

Application : Functional Assay

Description

The NF-kB Leeporter™ Luciferase Reporter cell line is a stably transfected RAW 264.7 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.

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.
- 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 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_2 incubator.

Leave the T25 flask in the incubator for 1~2 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. ***Note: RAW264.7 cells may not be detached well by trypsinization only. So you may need to use a cell scraper to harvest the trypsinized cells.***

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 NF-kB Leeporter™ – RAW264.7 cells to lipopolysaccharide (LPS) or various TLR ligands

1. Plate NF-kB Leeporter™ – RAW264.7 cells into a white solid-bottom 96-well microplate in 100 ul of growth medium

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at 1 x 10^5 cells/well and incubate cells at 37°C in a CO_2 incubator for 4-6 hours.

2. Stimulate cells with different concentrations of LPS (or various TLR ligands as noted in Figure 3), 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.

Commercial License Agreement is available for non-research/ non-inhouse/ commercial use if applicable. Please contact Abeomics (info@abeomics.com).

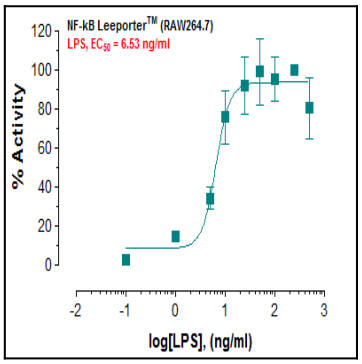


Fig-1: Induction of NF-kB activity by LPS (Cat. No. 15-1013) in NF-kB Leeporter™ - RAW264.7 cells.

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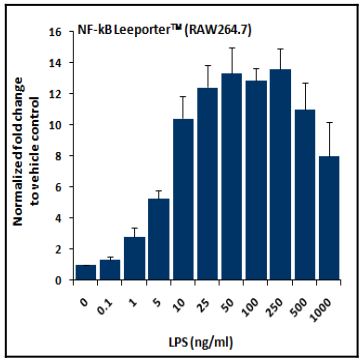


Fig-2: Induction of NF-kB activity by LPS (Cat. No. 15-1013) in NF-kB Leeporter™ - RAW264.7 cells.

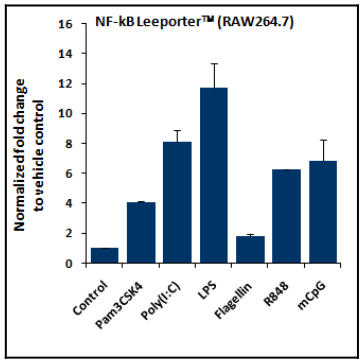


Fig-3: Induction of NF-kB activity by various TLR ligands in NF-kB Leeporter™ - RAW264.7 cells. Pam3CSK4 (10 ng/ml, Cat. No. 15-1011), Poly(I).Poly(C)-HMW (50 ug/ml, Cat. No. 15-1012), LPS (100 ng/ml, Cat. No. 15-1013), Flagellin (100 ng/ml, Cat. No. 15-1014), R848 (10 ug/ml, Cat. No. 15-1016), and CpG ODN 1826 (10 ug/ml, Cat. No. 15-1018).

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