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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.



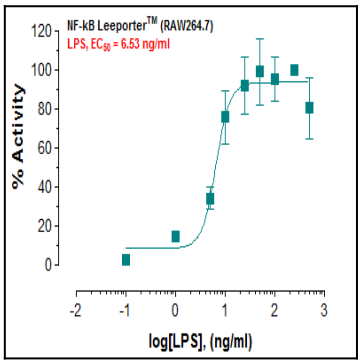


Fig-1: Induction of NF-kB activity by LPS (Cat. No. 15-1013) in NF-kB Leeporter™ - RAW264.7 cells.

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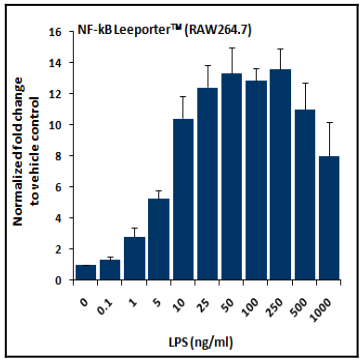


Fig-2: Induction of NF-kB activity by LPS (Cat. No. 15-1013) in NF-kB Leeporter™ - RAW264.7 cells.

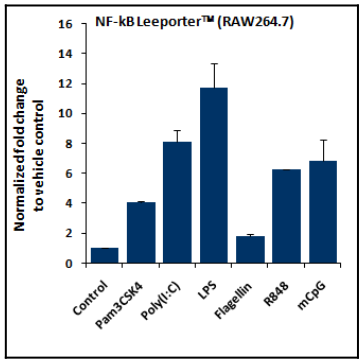


Fig-3: Induction of NF-kB activity by various TLR ligands in NF-kB Leeporter™ - RAW264.7 cells. Pam3CSK4 (10 ng/ml, Cat. No. 15-1011), Poly(I).Poly(C)-HMW (50 ug/ml, Cat. No. 15-1012), LPS (100 ng/ml, Cat. No. 15-1013), Flagellin (100 ng/ml, Cat. No. 15-1014), R848 (10 ug/ml, Cat. No. 15-1016), and CpG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