



NF- κ B Luciferase Reporter- RAW264.7 Cell Line

Catalog number: RC1000

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

NF- κ B Luciferase Reporter-RAW264.7 Cell Line

Catalog Number: RC1000, **Storage:** Immediately upon receipt, store in liquid nitrogen. (Ship on dry ice.)

Contents: Each vial contains $2 \sim 3 \times 10^6$ cells in 1 ml of 90% FBS + 10% DMSO.

Description: The NF- κ B 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- κ B response element. NF- κ B is a key transcription factor that is involved in immune and inflammatory responses, developmental processes, cellular growth and apoptosis.

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of NF- κ B

Application Details:

Application:

Monitor the NF- κ B signaling pathway.

Screen for activators or inhibitors of the NF- κ B signaling pathway.

Culture conditions:

Cells should be grown at 37°C with 5% CO₂ using DMEM medium supplemented with 10% FBS and 1% Pen/Strep, plus 3 μ g/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-CO₂ 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.

Functional validation:

A. Response of NF- κ B RAW264.7 cells to lipopolysaccharide (LPS)

1. Harvest NF- κ B RAW264.7 cells and seed cells into a white solid-bottom 96-well microplate in 100 μ l of growth medium at 8.5×10^4 cells/well.
2. Incubate cells at 37°C in a CO₂ incubator for overnight.
3. The next day, stimulate cells with different concentrations of LPS.
4. Incubate at 37°C in a CO₂ incubator for 6-16 hours.
5. Add 50 μ l of luciferase assay reagent per well.
6. Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer.

NF- κ B Luciferase Reporter-RAW264.7 Cell Line (RC1000) Images

Fig-1: Induction of NF- κ B activity by LPS in NF- κ B RAW264.7 cells.

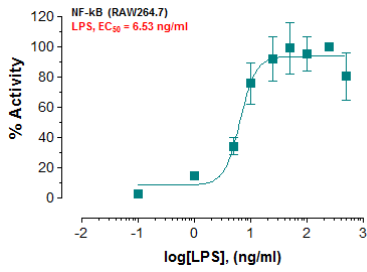


Fig-2: Induction of NF-kB activity by LPS in NF-kB Lipoporter™ - RAW264.7 cells.

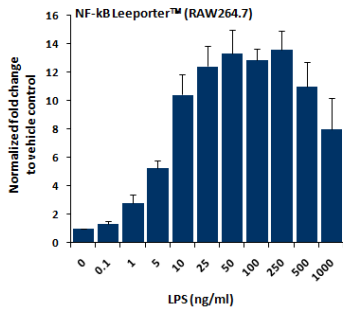
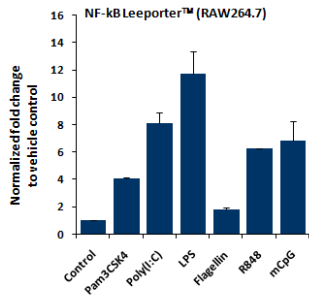


Fig-3: Induction of NF-kB activity by various TLR ligands in NF-kB Lipoporter™ - RAW264.7 cells. Pam3CSK4 (10 ng/ml), Poly(I).Poly(C)-HMW (50 ug/ml), LPS (100 ng/ml), Flagellin (100 ng/ml), R848 (10 ug/ml), and CpG ODN 1826 (10 ug/ml).



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