



IL-8 Luciferase Reporter- RAW264.7 Cell Line

Catalog number: RC1001

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

IL-8 Luciferase Reporter-RAW264.7 Cell Line

Catalog Number: RC1001, **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 IL-8 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 IL-8 promoter. IL-8 is one of the key proinflammatory chemokines or cytokines, which is produced by macrophages and other epithelial cells. Induction of IL-8 is associated with inflammation. The IL-8 induction by Toll-like receptor 4 (TLR4) ligand, LPS, is shown in Figure 1.

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of IL-8

Application Details:

Application:

Monitor the IL-8 induction activity.

Screen for activators or inhibitors of the IL-8 induction.

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 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-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. To achieve satisfactory results, cells should not be passaged over 16 times.

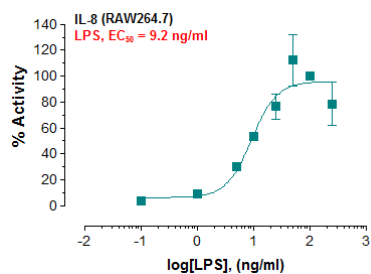
Functional validation:

A. Response of IL-8 RAW264.7 cells to lipopolysaccharide (LPS)

1. Harvest IL-8 RAW264.7 cells and seed cells into a white solid-bottom 96-well microplate in 100 µl 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 µl of luciferase assay reagent per well.
6. Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer.

IL-8 Luciferase Reporter-RAW264.7 Cell Line (RC1001) Images

Fig-1: Induction of IL-8 promoter activity by LPS in IL-8 RAW264.7 cells.



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