



TLR4/IL-8 Luciferase Reporter- HeLa Cell Line

Catalog number: RC1024

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

TLR4/IL-8 Luciferase Reporter-HeLa Cell Line

Catalog Number: RC1024, **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 TLR4/IL-8 Luciferase Reporter cell line is a stably transfected HeLa cell line which expresses human TLR4, MD-2 and CD14 as well Renilla luciferase reporter gene under the transcriptional control of the IL-8 promoter. IL-8 is one of the major pro-inflammatory cytokines induced by ligand (such as LPS)-mediated Toll-like receptor 4 (TLR4) activation. TLR4 is one of the key innate immune receptors, which is activated by LPS and can lead to sepsis upon dysregulation. The TLR4/IL-8 activation by LPS is shown in Figure 1.

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of TLR4/IL-8

Application Details:

Application:

Monitor the TLR4 signaling pathway activity. Screen for activators or inhibitors of the TLR4 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 1 µg/ml Puromycin, 5 µg/ml blasticidin and 500 µg/ml G418. 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, Blasticidin and G418, spin down cells, resuspend cells in pre-warmed growth medium without Puromycin, Blasticidin and G418, transfer resuspended cells to T25 flask and culture in 37°C-CO₂ incubator. Leave the T25 flask in the incubator for 1~3 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, Blasticidin and G418. Cells should be split before they reach complete confluence. 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 TLR4/IL-8 – HeLa cells to LPS. 1. Harvest TLR4/IL-8 – HeLa cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 5×10^4 cells/well. 2. Incubate cells at 37°C in a CO₂ incubator for overnight. 3. The next day, stimulate cells with various 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.

TLR4/IL-8 Luciferase Reporter-HeLa Cell Line (RC1024) Images

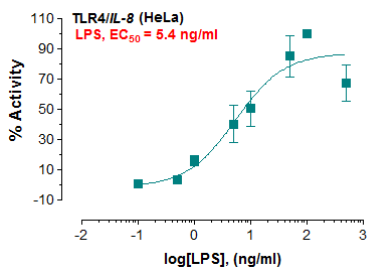


Fig-1: Induction of TLR4 activity by LPS in TLR4/IL-8 HeLa cells.

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