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14-124ACL: TLR4/IL-8 Leeporter™ Luciferase Reporter-HeLa Cell Line

Application : Functional Assay

Description

The TLR4/IL-8 Leeporter™ Luciferase Reporter cell line is a stably transfected HeLa cell line which expresses human TLR4, MD-2 and CD14 as well as 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.

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 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% 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 1 µg/ml of Puromycin, 5 ug/ml Blasticidin and 500 µg/ml G418 (Note: Puromycin, Blasticidin and G418 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, 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-CO_2 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. To achieve satisfactory results, cells should not be passaged over 16 times.

Functional validation:

For Research Use Only. Not for use in diagnostic/therapeutics procedures.

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A. Response of TLR4/IL-8 Leeporter™ – HeLa cells to LPS.

1. Harvest TLR4/IL-8 Leeporter™ – HeLa cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 5 x 10^4 cells/well.

2. Incubate cells at 37°C in a CO_2 incubator for overnight.

3. The next day, stimulate cells with various concentrations of LPS.

4. Incubate at 37°C in a CO_2 incubator for 16 hours.

5. Equilibrate the plate to room temperature for 10 minutes.

6. Add 50 µl of luciferase assay reagent (Abeomics, Cat #17-1101; Refer to the reagent datasheet for the detailed luciferase assay protocol) per well.

7. 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.

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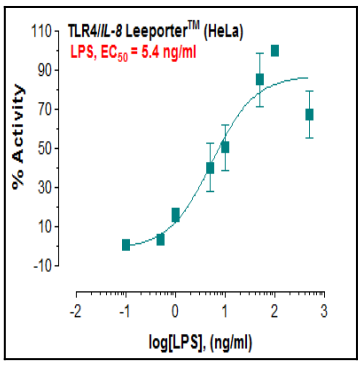


Fig-1: Induction of TLR4 activity by LPS in TLR4/IL-8 Leeporter™ - HeLa cells.