

## 14-124ACL: TLR4/IL-8 Reporter – HeLa Cell Line

**Application :** Functional Assay

### Description

The TLR4/IL-8 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.

### Product Info

<b>Amount :</b>	1 Vial
<b>Content :</b>	Each vial contains 2 ~ 3 x 10 <sup>6</sup> cells in 1 ml of 90% FBS + 10% DMSO.
<b>Storage condition :</b>	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<sub>2</sub> 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 µg/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<sub>2</sub> 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:

##### A. Response of TLR4/IL-8 Reporter™ – 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  $5 \times 10^4$  cells/well.
2. Incubate cells at 37°C in a CO<sub>2</sub> incubator for overnight.
3. The next day, stimulate cells with various concentrations of LPS.
4. Incubate at 37°C in a CO<sub>2</sub> 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:

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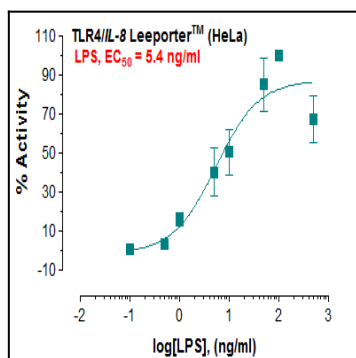


Fig-1: Induction of TLR4 activity by LPS in TLR4/IL-8 Leeporter™ – HeLa cells.