

## 14-106ACL: TNF-alpha LEEporter™ Luciferase Reporter-RAW264.7 Cell Line

**Application :** Functional Assay

### Description

The TNF-alpha LEEporter™ Luciferase Reporter cell line is a stably transfected RAW264.7 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the TNF-alpha promoter. Tumor necrosis factor-alpha (TNF-alpha) is one of the major proinflammatory cytokines and can induce systemic inflammation, apoptotic cell death, sepsis and cachexia. Dysregulation of TNF-alpha induction is often involved in various human diseases including inflammatory bowel disease, cancer and Alzheimer's disease. The TNF-alpha induction by lipopolysaccharide (LPS), the Toll-like receptor 4 (TLR4) ligand, is shown in Figure 1.

### Product Info

**Amount :** 1 Vial  
**Content :** Each vial contains  $2 \sim 3 \times 10^6$  cells in 1 ml of 90% FBS + 10% DMSO.  
**Storage condition :** Immediately upon receipt, store in liquid nitrogen.

### Application Note

#### Application:

- Monitor the TNF-alpha induction activity.
- Screen for activators or inhibitors of the TNF-alpha 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 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-CO<sub>2</sub> 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 TNF-alpha LEEporter™ – RAW264.7 cells to lipopolysaccharide (LPS).

1. Plate TNF-alpha LEEporter™ – RAW264.7 cells into a white solid-bottom 96-well microplate in 100  $\mu$ l of growth medium at  $1 \times 10^5$  cells/well and incubate cells at 37°C in a CO<sub>2</sub> incubator for 4-6 hours.
2. Stimulate cells with different concentrations of LPS and incubate cells at 37°C in a CO<sub>2</sub> incubator for 16 hours.
3. Equilibrate the plate to room temperature for 10 minutes.
4. Add 50  $\mu$ l of luciferase assay reagent (Abeomics, Cat #17-1101; Refer to the reagent datasheet for the detailed luciferase assay protocol) per well.
5. 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.**

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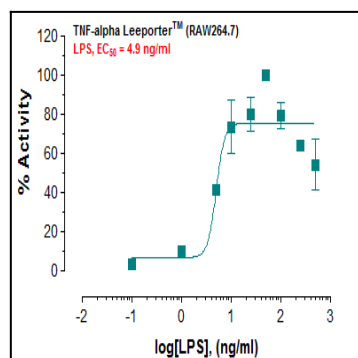


Fig-1: Induction of TNF-alpha promoter activity by LPS in TNF-alpha LEEporter™ - RAW264.7 cells.