

## 14-116ACL: IL-6 LEEPORTER™ Luciferase Reporter-NIH 3T3 Cell Line

**Application :** Functional Assay

### Description

The IL-6 LEEPORTER™ Luciferase Reporter cell line is a stably transfected NIH 3T3 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the IL-6 promoter. As a pleiotropic cytokine, interleukin 6 (IL-6) has pro- and anti-inflammatory roles which is not only involved in normal functions of the immune system, hematopoiesis and metabolism but also involved in the pathogenesis of metabolic and cardiovascular diseases. IL-6 gene induction is generally regulated by several transcription factors that activate the consensus sequences in the IL-6 promoter region, which include AP-1, C/EBP-beta and NF-kB in response to various proinflammatory cytokines, growth factors, and pathogen-associated molecular patterns such as Toll-like receptor (TLR) ligands. The IL-6 induction by lipopolysaccharide (LPS), the TLR4 ligand as well as by proinflammatory cytokines, IL-6 and IL-17A is shown in Figures 1 through 3.

### 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 IL-6 induction activity.
- Screen for activators or inhibitors of the IL-6 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 1~3 days without disturbing or changing the medium until cells completely recover viability and become adherent. Once cells are between 80~90% adherent, remove growth medium and passage the cells through trypsinization and centrifugation. At first passage, switch to growth medium containing Puromycin.

**Note: NIH 3T3 cells should be split before they reach 90% confluence; otherwise, they become self-lifted and aggregate irreversibly. Precoating the cell plates with 0.2% gelatin may prevent NIH 3T3 cells from self-lifting. During cell trypsinization, cells covered enough with trypsin-EDTA solution should be stayed at 37°C for 10 min without agitation.**

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-6 LEEporter™ – NIH 3T3 cells to lipopolysaccharide (LPS).

1. Harvest IL-6 LEEporter™ – NIH 3T3 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 4-6 hours.
3. 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.

##### B. Response of IL-6 LEEporter™ – NIH 3T3 cells to IL-6 or IL-17A.

1. Harvest IL-6 LEEporter™ – NIH 3T3 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 4-6 hours.
3. Stimulate cells with various concentrations of IL-6 or IL-17A.
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) 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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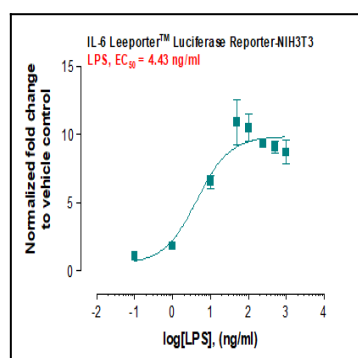


Fig-1: Induction of IL-6 promoter activity by LPS in IL-6 LEEporter™ – NIH 3T3 cells.

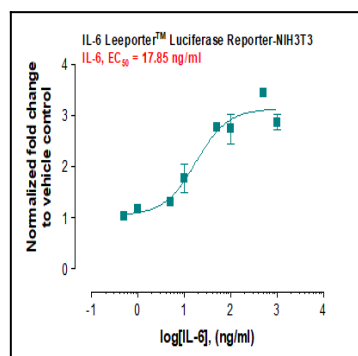


Fig-2: Induction of IL-6 promoter activity by IL-6 in IL-6 LEEporter™ – NIH 3T3 cells.

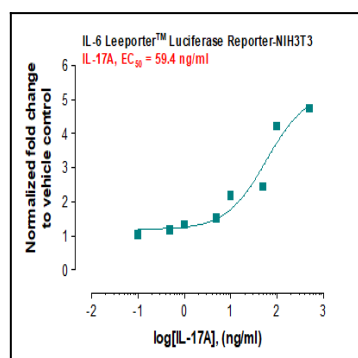


Fig-3: Induction of IL-6 promoter activity by IL-17A in IL-6 LEEporter™ – NIH 3T3 cells.