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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

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 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_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 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_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 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 irrevisibly. 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.

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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 x 10^4 cells/well.

2. Incubate cells at 37°C in a CO_2 incubator for 4-6 hours.

3. 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.

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 x 10^4 cells/well.

2. Incubate cells at 37°C in a CO_2 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_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) 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

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or otherwise transfer this product to a third party.

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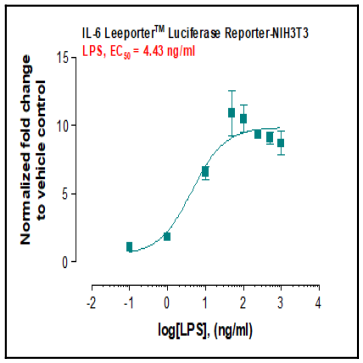

Fig-1: Induction of IL-6 promoter activity by LPS in IL-6 Leeporter™ - NIH 3T3 cells.

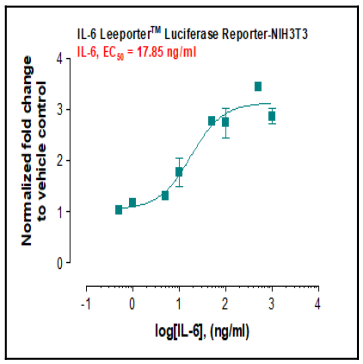

Fig-2: Induction of IL-6 promoter activity by IL-6 in IL-6 Leeporter™ - NIH 3T3 cells.

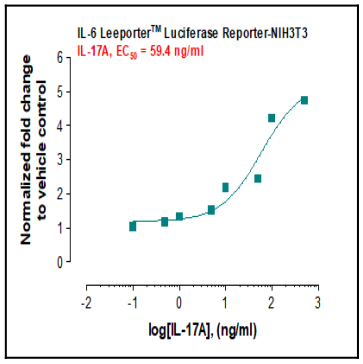

Fig-3: Induction of IL-6 promoter activity by IL-17A in IL-6 Leeporter™ - NIH 3T3 cells.