



IL-6 Luciferase Reporter-NIH 3T3 Cell Line

Catalog number: RC1016

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

IL-6 Luciferase Reporter-NIH 3T3 Cell Line

Catalog Number: RC1016, **Storage:** Immediately upon receipt, store in liquid nitrogen. (Ship on dry ice.)

Contents: Each vial contains $2 \sim 3 \times 10^6$ cells in 1 ml of 90% FBS + 10% DMSO.

Description: The IL-6 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, is shown in Figure 1.

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of IL-6

Application Details:

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% CO₂ using DMEM medium supplemented with 10% FBS and 1% Pen/Strep, plus 3 µg/ml of Puromycin. 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-CO₂ 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 assay plates with 0.1% gelatin may prevent NIH 3T3 cells from self-lifting. 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.

Functional validation:

A. Response of IL-6 NIH 3T3 cells to lipopolysaccharide (LPS). 1. Harvest IL-6 NIH 3T3 cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 5×10^4 cells/well. 2. Incubate cells at 37°C in a CO₂ incubator for overnight. 3. The next day, stimulate cells with various concentrations of LPS. 4. Incubate at 37°C in a CO₂ incubator for 6-16 hours. 5. Add 50 µl of luciferase assay reagent per well. 6. Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer.

IL-6 Luciferase Reporter-NIH 3T3 Cell Line (RC1016) Images

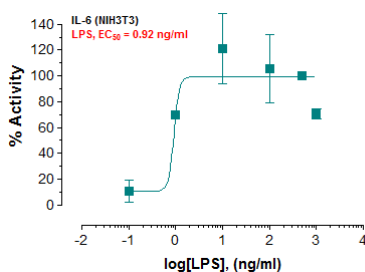


Fig-1: Induction of IL-6 promoter activity by LPS in IL-6 NIH 3T3 cells.

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