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TNF-beta Luciferase Reporter-RAW264.7 Cell Line

Catalog number: RC1008

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

TNF-beta Luciferase Reporter-RAW264.7 Cell Line

Catalog Number: RC1008, **Storage:** Immediately upon receipt, store in liquid nitrogen. (Ship on dry ice.)
Contents: Each vial contains 2 ~ 3 x 10^6 cells in 1 ml of 90% FBS + 10% DMSO.
Description: The TNF-beta Luciferase Reporter cell line is a stably transfected RAW264.7 cell line which expresses Renilla luciferase reporter gene

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under the transcriptional control of the TNF-beta promoter. Tumor necrosis factor-beta (TNF-beta) one of the major proinflammatory cytokines, which is produced predominantly by mitogen-stimulated T lymphocytes and leukocytes. TNF-beta is involved in the regulation of various biological processes such as cell proliferation, differentiation and apoptosis. TNF-beta gene polymorphisms are also significantly associated with disease susceptibility and phenotype in patients with psoriatic arthritis. The TNF-beta induction by lipopolysaccharide (LPS), the Toll-like receptor 4 (TLR4) ligand, is shown in Figure 1.

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of TNF-beta

Application Details:

Application:

Monitor the TNF-beta induction activity.Screen for activators or inhibitors of the TNF-beta 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 1~2 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. 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 TNF-beta RAW264.7 cells to lipopolysaccharide (LPS). 1. Harvest TNF-beta RAW264.7 cells and seed cells into a white solid-bottom 96-well microplate in 100 μl of growth medium at 8.5 x 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.

TNF-beta Luciferase Reporter-RAW264.7 Cell Line (RC1008) Images

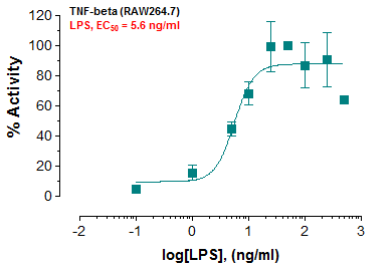


Fig-1: Induction of TNF-beta promoter activity by LPS in TNF-beta RAW264.7 cells.

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