



iNOS Luciferase Reporter- RAW264.7 Cell Line

Catalog number: RC1015

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

iNOS Luciferase Reporter-RAW264.7 Cell Line

Catalog Number: RC1015, **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 iNOS Luciferase Reporter cell line is a stably transfected RAW264.7 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the iNOS promoter. Inducible nitric oxide synthase (iNOS) is an inducible enzyme that catalyzes the production of nitric oxide (NO) from L-arginine. NO is one of the smallest signaling molecules that can diffuse into the cell and is involved in various physiological functions, pathogenesis of septic shock, many diseases associated with autoimmunity, and tumorigenesis. iNOS gene is generally known to be induced by various proinflammatory cytokines and pathogen-associated molecular patterns such as Toll-like receptor (TLR) ligands. The iNOS induction by lipopolysaccharide (LPS), the TLR4 ligand, is shown in Figure 1.

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of iNOS

Application Details:

Application:

Monitor the iNOS induction activity. Screen for activators or inhibitors of the iNOS 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 iNOS RAW264.7 cells to lipopolysaccharide (LPS). 1. Harvest iNOS RAW264.7 cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 8.5×10^4 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.

iNOS Luciferase Reporter-RAW264.7 Cell Line (RC1015) Images

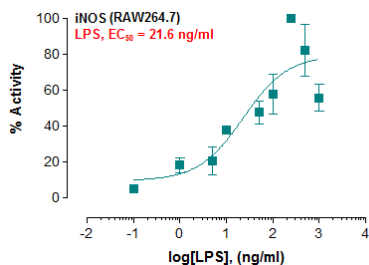


Fig-1: Induction of iNOS promoter activity by LPS in iNOS RAW264.7 cells.

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