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14-115ACL: iNOS Reporter – RAW264.7 Cell Line

Application : Functional Assay

Description

The iNOS 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 TLR ligands. The iNOS induction by various Toll-like receptor (TLR) ligands as well as phorbol 12-myristate 13-acetate is shown in Figure 1.

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 iNOS induction activity.
- Screen for activators or inhibitors of the iNOS 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 1~2 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. ***Note: RAW264.7 cells may not be detached well by trypsinization only. So you may need to use a cell scraper to harvest the trypsinized cells.***

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:

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A. Response of iNOS Leeporter™ – RAW264.7 cells to lipopolysaccharided (LPS).

1. Plate iNOS Leeporter™ – RAW264.7 cells into a white solid-bottom 96-well microplate in 100 ul of growth medium at 1 x 10^5 cells/well and incubate cells at 37°C in a CO_2 incubator for 4-6 hours.

2. Stimulate cells with different concentrations of LPS and incubate cells at 37°C in a CO_2 incubator for 16 hours. ,

3. Equilibrate the plate to room temperature for 10 minutes.

4. Add 50 µl of luciferase assay reagent (Abeomics, Cat #17-1101; Refer to the reagent datasheet for the detailed luciferase assay protocol) per well.

5. 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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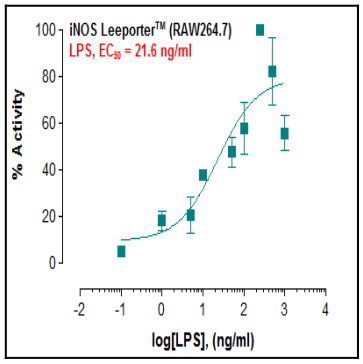

Fig-1: Induction of iNOS promoter activity by LPS in iNOS Leeporter™ – RAW264.7 cells.