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TLR3/IFNB Luciferase Reporter-HEK293 Cell Line

Catalog number: RC1022

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

TLR3/IFNB Luciferase Reporter-HEK293 Cell Line

Catalog Number: RC1022, **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 TLR3/IFNB Luciferase Reporter cell line is a stably transfected HEK 293 cell line which expresses full-length human Toll-like

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receptor 3 (TLR3) and Renilla luciferase reporter gene under the transcriptional control of the human IFN-beta (IFNB) promoter. Functional activity of the cell line has been validated by TLR3 ligand assay (Figure 1), in which upon activation by poly (I:C), TLR3 quickly initiates TRIF-dependent signaling pathway and mediates nuclear translocation of IRF3 that induces IFNB promoter activity.The TLR3/IFNB activation by poly (I:C) is shown in Figure 1.

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of TLR3/IFNB

Application Details:

Application:

Monitor the TLR3 signaling pathway activity.Screen for activators or inhibitors of the TLR3 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 2 µg/ml Puromycin and 5 µg/ml Blasticidin. 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 and Blasticidin, spin down cells, resuspend cells in pre-warmed growth medium without Puromycin and Blasticidin, transfer resuspended cells to T25 flask and culture in 37°C-CO2 incubator. Leave the T25 flask in the incubator for 2~4 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 and Blasticidin. 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 TLR3/IFNB – HEK293 cells to Poly (I:C).1. Harvest TLR3/IFNB – HEK293 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 CO2 incubator for overnight. 3. The next day, stimulate cells with various concentrations of poly (I:C). 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.

TLR3/IFNB Luciferase Reporter-HEK293 Cell Line (RC1022) Images

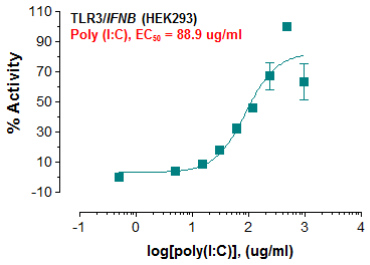


Fig-1: Induction of TLR3 activity by poly(I:C) in TLR3/IFNBr HEK293 cells.

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