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TLR8/NF-kB Luciferase Reporter-HEK293 Cell Line

Catalog number: RC1030

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

TLR8/NF-kB Luciferase Reporter-HEK293 Cell Line

Catalog Number: RC1030, **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 TLR8/NF-kB Luciferase Reporter cell line is a stably transfected HEK 293 cell line which expresses full-length human Toll-like

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receptor 8 (TLR8) and Renilla luciferase reporter gene under the transcriptional control of the NF-kB response element. TLR8 is one of the key innate immune receptors. Functional activity of the cell line has been validated by TLR8 ligand assay, in which upon activation by R848, TLR8 quickly initiates downstream signaling pathway and mediates nuclear translocation of NF-kB (Figure 1).

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of TLR8/NF-kB

Application Details:

Application:

Monitor the TLR8 signaling pathway activity.Screen for activators or inhibitors of the TLR8 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 of Puromycin and 5 µg/ml of 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 TLR8/NF-kB – HEK293 cells to R848.1. Harvest TLR8/NF-kB – 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 R848. 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.

TLR8/NF-kB Luciferase Reporter-HEK293 Cell Line (RC1030) Images

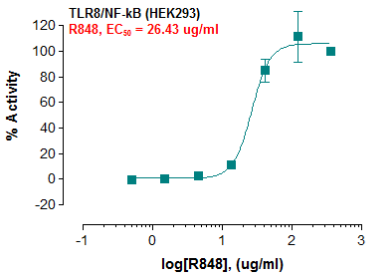


Fig-1: Induction of TLR8 activity by R848 in TLR8/NF-kB HEK293 cells.

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