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14-701ACL: TLR3/NF-kB Leeporter™ GFP Reporter-HEK293 Cell Line

Application : Functional Assay

Description

The TLR3/NF-kB Leeporter™ GFP Reporter cell line is a stably transfected HEK293 cell line, which expresses full-length human Toll-like receptor 3 (TLR3) and enhanced green fluorescent protein (eGFP) reporter gene under the transcriptional control of the NF-kB response element. Functional activity of the cell line has been validated by TLR3 ligand assay, in which upon activation by poly (I:C), TLR3 quickly initiates TRIF-dependent signaling pathway and mediates nuclear translocation of NF-kB (Figures 1 and 2).

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 TLR3 signaling pathway.
- Screen for activators or inhibitors of the TLR3 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 and 15 µg/ml of Blasticidin (Note: Puromycin and Blasticidin 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 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-CO_2 incubator.

Leave the T25 flask in the incubator for 1~3 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. To achieve satisfactory results, cells should not be passaged over 16 times.

Functional validation:

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A. Response of TLR3/NF-kB Leeporter™ GFP reporter – HEK293 cells to Poly(I:C).

1. Harvest TLR3/NF-kB Leeporter™ GFP reporter – HEK293 cells and seed cells into a tissue culture plate (e.g. 96-well plate in 100 ul of growth medium at 5 x 10^4 cells/well, 24-well plate in 500 ul of growth medium at 2.5 x 10^5 cells/well, 12-well plate in 1 ml of growth medium at 5 x 10^5 cells/well, or 6-well plate in 2 ml of growh medium at 1 x 10^6 cells/well.

2. Incubate cells at 37°C in a CO_2 incubator for overnight.

3. The next day, stimulate cells with different concentrations of Poly(I:C) (Abeomics, Cat. No.: 15-1012).

4. Incubate at 37°C in a CO_2 incubator for 16 hours.

5.Analyze cells through fluorescence microscopy or flow cytometry.

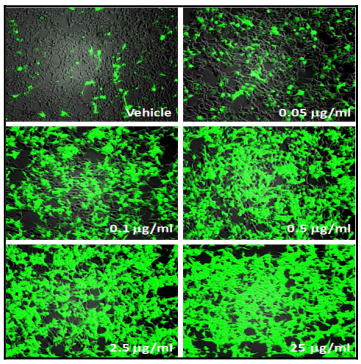

Fig-1: Induction of TLR3 activity by Poly(I:C) in TLR3/NF-kB Leeporter™ GFP reporter-HEK293 cells was analyzed by fluorescence microscopy. Poly(I:C) was treated at 0.05, 0.1, 0.5, 2.5 and 25 ug/ml as noted.

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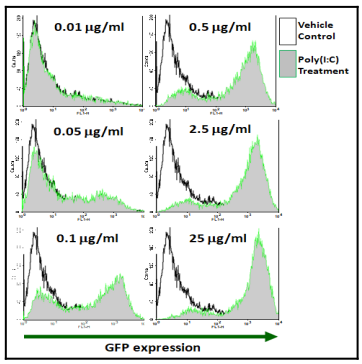

Fig-2: Induction of TLR3 activity by Poly(I:C) in TLR3/NF-kB Leeporter™ GFP reporter-HEK293 cells was analyzed by flow cytometry. Poly(I:C) was treated at 0.01, 0.05, 0.1, 0.5, 2.5 and 25 ug/ml as noted. Vehicle control (Black line, empty); Poly(I:C) treatment (Green line, grey-filled).