BOSTER BIOLOGICAL TECHNOLOGY
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BOSTER
antibody and ELISA experts

MDA5/NF-kB Luciferase Reporter-HEK293T Cell Line

Catalog number: RC1021

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

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Catalog Number: RC1021, **Storage:** Immediately upon receipt, store in liquid nitrogen. (Ship on dry ice.)

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Contents: Each vial contains 2 ~ 3 x 10^6 cells in 1 ml of 90% FBS + 10% DMSO.
Description: The MDA5 Luciferase Reporter cell line is a stably transfected HEK 293T cell line which expresses human melanoma differentiation-associated protein-5 (MDA5) and Renilla luciferase reporter gene under the transcriptional control of an NF-kB response element. As a dsRNA helicase enzyme, MDA5 is encoded by the IFIH1 gene. MDA5 is one of the RIG-I-like receptors (RLRs) that are a family of DExD/H box RNA helicases including MDA5, RIG-I and LPG2, which play a major role in pathogen sensing of RNA virus infection to initiate and modulate antiviral immunity. RLR expression is typically maintained at low levels in resting cells but is greatly increased during inflammation, specifically with IFN exposure and after virus infection. MDA5 detects cytoplasmic dsRNA generated during viral replication unlike Toll-like receptor 3 (TLR3) which can detect phagocytosed dsRNA in endosomes. MDA5 also responds to poly (I:C), the synthetic analog of viral dsRNA. The MDA5 activation by poly (I:C) is shown in Figure 1.
Applications: Functional Assay
Application Notes: Functional Assay, detecting the transcriptional activity of MDA5/NF-kB
Application Details: Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.
If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.
Some PubMed article(s) citing the expression level of this target are as follows:
Boster Bio's internal QC testing used:

Application:

Monitor the MDA5 signaling pathway activity.Screen for activators or inhibitors of the MDA5 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 MDA5 HEK293T cells to Poly (I:C).1. Harvest MDA5 HEK293T 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.

MDA5/NF-kB Luciferase Reporter-HEK293T Cell Line (RC1021) Images

Fig-1: Induction of MDA5 activity by poly(I:C) prepacked with lipofectamine in MDA5 HEK293T cells.

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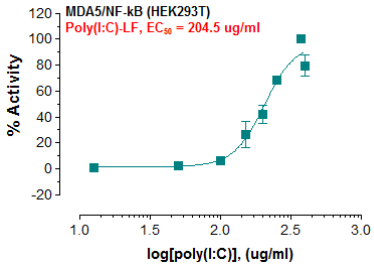

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