

MDA5/NF-kBT cell line

MDA5/NF-kB LEEPORTER Luciferase Reporter-HEK293T Cell Line

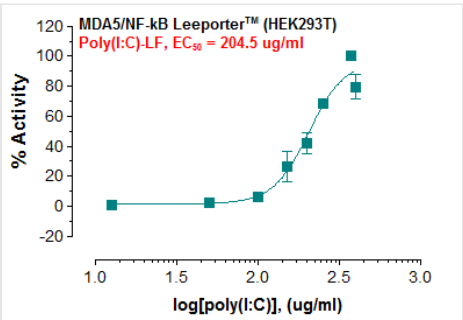
CATALOG # AAA14874	APPLICATIONS Functional Assay
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PRODUCT SYNONYMS

MDA5/NF-kBT; MDA5/NF-kB LEEPORTER Luciferase Reporter-HEK293T Cell Line; MDA5/NF-kBT cell line

PRODUCT SPECIFICATIONS

Applicable Applications for MDA5/NF-kBT cell line	FA (Functional Assay)
Application Notes	Monitor the MDA5 signaling pathway activity. Screen for activators or inhibitors of the MDA5 signaling pathway.
Content	Each vial contains 2 ~ 3 x 10 ⁶ cells in 1 ml of 90% FBS + 10% DMSO.
Culture conditions	Cells should be grown at 37 degree C with 5% CO₂ using DMEM medium (w/ L-Glutamine, 4.5g/L Glucose and Sodium Pyruvate) supplemented with 10% heat-inactivated FBS and 1% Pen/Strep, plus 2 ug/ml of Puromycin and 5 ug/ml 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 degree C water-bath, transfer to a tube containing 10 ml of growth medium without Puromycin and Blasticidin, spin down cells, resuspend cells in prewarmed growth medium without Puromycin and Blasticidin, transfer resuspended cells to T25 flask and culture in 37 degree C CO₂ 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 ratio = 1:10 to 1:20 weekly. To achieve satisfactory results, cells should not be passaged over 16 times.
Functional validation	<u>A. Response of MDA5 LEEPORTER™ – HEK293T cells to Poly(I:C).</u> 1. Harvest MDA5 LEEPORTER™ – HEK293T cells and seed cells into a white solid-bottom 96-well microplate in 100 ul of growth medium at 5 x 10⁴ cells/well. 2. Incubate cells at 37 degree C in a CO₂ incubator for overnight. 3. The next day, stimulate cells with various concentrations of the poly(I:C) packed with lipofectamine 2000, which was prepared as follows: i) A 50 ul of 10 mg/ml poly(I:C) (= 500 ug total) is preincubated in 50 ul Opti-MEM (Life Technologies) for 5 min. ii) Similarly, a 20 ul Lipofectamine 2000 (Life Technologies) is preincubated in 80 ul Opti-MEM for 5 min. iii) After 5 min, they are combined together as a total volume of 200 ul and further incubated for 20 min at room temperature. iv) The poly(I:C) packed with Lipofectamine 2000 (A 200 ul total at 2.5 mg/ml) is then used to stimulate cells. 4. Incubate at 37 degree C in a CO₂ incubator for 16 hours. 5. Equilibrate the plate to room temperature for 10 minutes. 6. Add 50 ul of luciferase assay reagent (Cat # Refer to the reagent datasheet for the detailed luciferase assay protocol) per well. 7. Read the plate in 1-5 minutes to measure luminescence using a microplate luminometer.
Preparation and Storage	Immediately upon receipt, store in liquid nitrogen.



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

ADDITIONAL INFORMATION

Related Product Information for MDA5/NF-kBT cell line	The MDA5 Leeporter™ 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- κ B 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.
Product Categories/Family for MDA5/NF-kBT cell line	Reporter Cell Lines

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.