Revision 1

MDA-5 (D74E4) Rabbit mAb

#5321 Store at -20C

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP	H M	Endogenous	135	Rabbit IgG	#Q9BYX4	64135

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

MDA-5 (D74E4) Rabbit mAb detects endogenous levels of total MDA-5 protein.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Arg470 of human MDA-5.

Background

Antiviral innate immunity depends on the combination of parallel pathways triggered by virus detecting proteins in the Toll-like receptor (TLR) family and RNA helicases, such as Rig-I (retinoic acid-inducible gene I) and MDA-5 (melanoma differentiation-associated antigen 5), which promote the transcription of type I interferons (IFN) and antiviral enzymes (1-3). TLRs and helicase proteins contain sites that recognize the molecular patterns of different virus types, including DNA, single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), and glycoproteins. These antiviral proteins are found in different cell compartments; TLRs (i.e. TLR3, TLR7, TLR8, and TLR9) are expressed on endosomal membranes and helicases are localized to the cytoplasm. Rig-I expression is induced by retinoic acid, LPS, IFN, and viral infection (4,5). Both Rig-I and MDA-5 share a DExD/H-box helicase domain that detects viral dsRNA and two amino-terminal caspase recruitment domains (CARD) that are required for triggering downstream signaling (4-7). Rig-I binds both dsRNA and viral ssRNA that contains a 5'-triphosphate end not seen in host RNA (8,9). Though structurally related, Rig-I and MDA-5 detect a distinct set of viruses (10,11). The CARD domain of the helicases, which is sufficient to generate signaling and IFN production, is recruited to the CARD domain of the MAVS/VISA/Cardif/IPS-1 mitochondrial protein, which triggers activation of NF-κB, TBK1/IKKε, and IRF-3/IRF-7 (12-15). MDA-5 (16,17), also named Ifih1 (interferon induced with helicase C domain 1), RH116 (RNA helicase-DEAD box protein 116) (18), or Helicard (19) is found to be induced by interferon. During apoptosis, MDA-5 is cleaved by caspases, separating the helicase and CARD domains (19). MDA-5 is uniquely activated by picornavirus (20) and measles virus (21).

Background References

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Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **IP:** Immunoprecipitation

Cross-Reactivity Key

H: Human **M:** Mouse

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