Store at -20°C

#50907

Phospho-STING (Ser366) (E9A9K) Rabbit mAb

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Entrez-Gene ID #340061
UniProt ID #Q86WV6

New 08/19

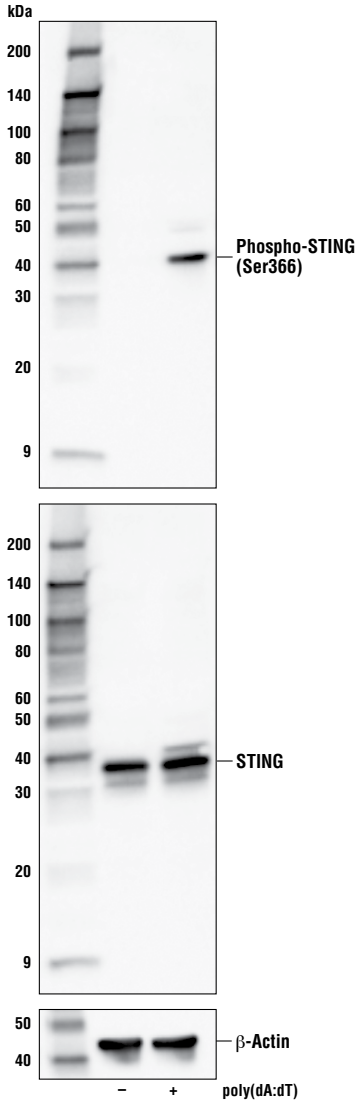
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, F Endogenous	H	40 kDa	Rabbit IgG**

Background: Stimulator of interferon genes (STING, TMEM173, MITA) is a transmembrane adaptor protein that is a critical component of the cellular innate immune response to pathogenic cytoplasmic DNA (1,2). STING is a ubiquitously expressed protein found predominantly in the ER (1). The enzyme cGAMP synthase (cGAS) produces the second messenger cyclic-GMP-AMP (cGAMP) in response to cytoplasmic DNA (3,4). cGAMP binds and activates STING (3,4). In addition, detection of cytoplasmic DNA by nucleic acid sensors, including DDX41 or IFI16, results in STING activation (5,6). Following activation, STING translocates with TBK1 to perinuclear endosomes (7). The TBK1 kinase phosphorylates and activates interferon regulatory factors (IRFs) and NF-κB, which leads to the induction of type I interferon and other immune response genes (1,2,7). Following activation and trafficking, STING is phosphorylated by ULK1 at Ser366 (Ser365 in mouse), which leads to STING inactivation and eventually lysosomal degradation (8).

Specificity/Sensitivity: Phospho-STING (Ser366) (E9A9K) Rabbit mAb recognizes endogenous levels of STING protein only when phosphorylated at Ser366.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser366 of human STING protein.



Western blot analysis of extracts from THP-1 cells differentiated with TPA (12-O-Tetradecanoylphorbol-13-Acetate) #4174 (80 nM, 16 hr) and then untransfected (-) or transfected with poly(dA:dT) (5 µg/mL, 3 hr; +) using Phospho-STING (Ser366) (E9A9K) Rabbit mAb (upper), STING (D2P2F) Rabbit mAb #13647 (middle), or β-Actin (D6A8) Rabbit mAb #8457 (lower).

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.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (IF-IC)	1:1600-1:3200
Fixative:	4% Formaldehyde
Permeabilization:	0.3% Triton X-100
Flow Cytometry	1:800-1:1600

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Background References:

(1) Ishikawa, H. and Barber, G.N. (2008) *Nature* 455, 674-8.
(2) Zhong, B. et al. (2008) *Immunity* 29, 538-50.
(3) Sun, L. et al. (2013) *Science* 339, 786-91.
(4) Wu, J. et al. (2013) *Science* 339, 826-30.
(5) Zhang, Z. et al. (2011) *Nat Immunol* 12, 959-65.
(6) Unterholzner, L. et al. (2010) *Nat Immunol* 11, 997-1004.
(7) Ishikawa, H. et al. (2009) *Nature* 461, 788-92.
(8) Konno, H. et al. (2013) *Cell* 155, 688-98.

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

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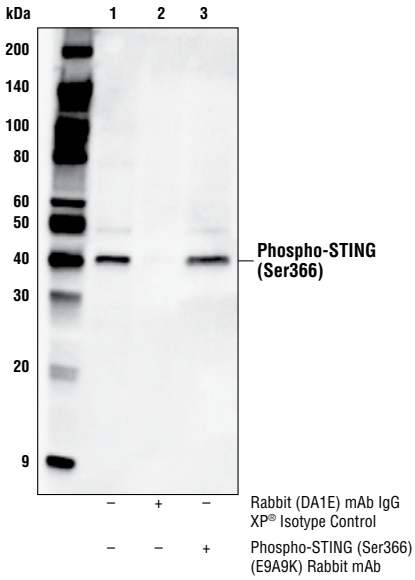
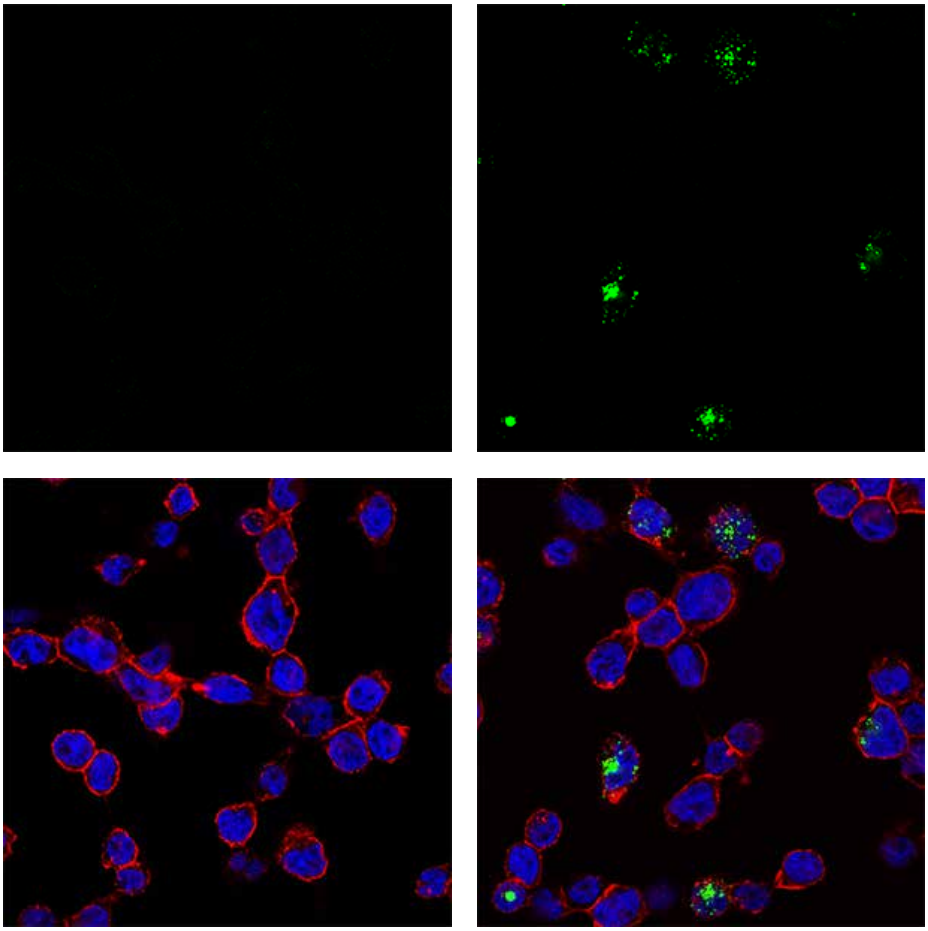
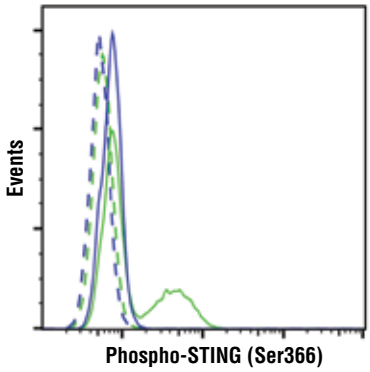
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Immunoprecipitation of Phospho-STING (Ser366) protein from THP-1 extracts differentiated with TPA (12-O-Tetradecanoylphorbol-13-Acetate) #4174 (80 nM, 16 hr) and then transfected with poly(dA:dT) (5 ug/mL, 3 hr). Lane 1 is 10% input, lane 2 is Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is Phospho-STING (Ser366) (E9A9K) Rabbit mAb. Western blot analysis was performed using Phospho-STING (Ser366) (E9A9K) Rabbit mAb. Mouse Anti-rabbit IgG (Conformation Specific) (L27A9) mAb #3678, followed by Anti-mouse IgG, HRP-linked Antibody #7076 were used as the secondary antibodies.



Confocal immunofluorescent analysis of THP-1 cells differentiated with TPA (12-O-Tetradecanoylphorbol-13-Acetate) #4174 (80 nM, 16 hr) and then mock-transfected (left) or transfected with poly(dA:dT) (5 µg/mL, 3 hr; right) using Phospho-STING (Ser366) (E9A9K) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Samples were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).



Flow cytometric analysis of THP-1 cells differentiated with TPA (12-O-Tetradecanoylphorbol-13-Acetate) #4174 (80 nM, 24 hrs), and then untransfected (blue) or transfected with poly(dA:dT) (5 ug/mL, 3 hr; green), using Phospho-STING (Ser366) (E9A9K) Rabbit mAb (solid lines) or a concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

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