

# PRMT1 (F339) Antibody

✓ 100 µl  
(10 Western mini-blots)

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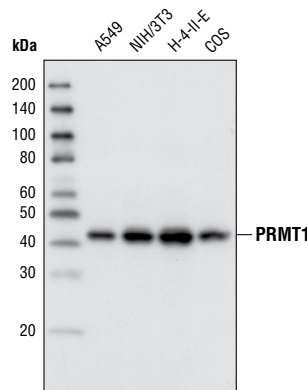
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New 03/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity	Molecular Wt.	Source
W	H, M, R, Mk, (B)	41 kDa	Rabbit

**Background:** Protein arginine N-methyltransferase 1 (PRMT1) is a member of the protein arginine N-methyltransferase (PRMT) family of proteins, which catalyze the transfer of a methyl group from S-adenosylmethionine (AdoMet) to a guanidine nitrogen of arginine (1). There are two types of PRMT proteins. While both types catalyze the formation of mono-methyl arginine, Type I PRMTs (PRMT1, 3, 4, and 6) add an additional methyl group to produce asymmetric di-methyl arginine and Type II PRMTs (PRMT 5 and 7) produce symmetric di-methyl arginine (1). Mono-methyl arginine, but not di-methyl arginine, can be converted to citrulline through deimination performed by enzymes such as PADI4 (2). Most of the PRMTs, including PRMT1, methylate arginine residues found within glycine-arginine rich (GAR) domains of proteins, such as RGG, RG, and RXR repeats (1). However, PRMT4/CARM1 and PRMT5 instead methylate arginine residues within PGM (proline-, glycine-, methionine-rich) motifs (3). PRMT1 methylates Arg3 of histone H4 and cooperates synergistically with p300/CBP to enhance transcriptional activation by nuclear receptor proteins (4,5,6). In addition, PRMT1 methylates many non-histone proteins, including the orphan nuclear receptor HNF4 (6), components of the heterogeneous nuclear ribonucleoprotein (hnRNP) particle (7), the RNA binding protein Sam68 (8), interleukin enhancer-binding factor 3 (ILF3) (9) and interferon- $\alpha$  and  $\beta$  receptors (10), suggesting additional functions in transcriptional regulation, mRNA processing and signal transduction. Alternative mRNA splicing results in three enzymatically active isoforms of PRMT1 protein that differ in their amino terminal regions (11). PRMT1 is localized in the nucleus or cytoplasm, depending on cell type (12,13) and appears in many distinct protein complexes. ILF3, TIS21 and the leukemia-associated BTG1 proteins bind to PRMT1 and regulate its methyltransferase activity (9,14).



Western blot analysis of cell lysates from A549, NIH/3T3, H-4-II-E and COS cells using PRMT1 Antibody.

**Specificity/Sensitivity:** PRMT1 (F339) Antibody detects endogenous levels of total PRMT1 protein (all three isoforms). The antibody does not cross-react with other PRMT proteins.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to the carboxy terminus of human PRMT1. Antibodies are purified by protein A and peptide affinity chromatography.

**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.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

**Recommended Antibody Dilutions:**

Western blotting 1:1000

**Companion Products:**

Acetyl-Histone H4 (Lys8) Antibody #2594

Acetyl-Histone H4 (Lys12) Antibody #2591

Histone H4 Antibody #2592

IFN- $\alpha$  (8C21) Mouse mAb #3115

IFN- $\alpha$  (6B18) Mouse mAb #3110

PRMT1 (A33) Antibody #2449

PRMT4/CARM1 Antibody #4438

Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

Anti-rabbit IgG, HRP-linked Antibody #7074

Prestained Protein Marker, Broad Range (Premixed Format) #7720

Biotinylated Protein Ladder Detection Pack #7727

20X LumiGLO® Reagent and 20X Peroxide #7003

**Background References:**

- Bedford, M.T. and Richard, S. (2005) *Mol. Cell* 18, 263–272.
- Wang, Y. et al. (2004) *Science* 306, 279–283.
- Cheng, D. et al. (2007) *Mol. Cell* 25, 71–83.
- Wang, H. et al. (2001) *Science* 293, 853–857.
- Strahl, B.D. et al. (2001) *Curr. Biol.* 11, 996–1000.
- Barrero, M.J. and Malik, S. (2006) *Mol. Cell* 24, 233–243.
- Nichols, R.C. et al. (2000) *Exp. Cell Res.* 256, 522–532.
- Côté, J. et al. (2003) *Mol. Biol. Cell* 14, 274–287.
- Tang, J. et al. (2000) *J. Biol. Chem.* 275, 19866–19876.
- Abramovich, C. et al. (1997) *EMBO J.* 16, 260–266.
- Scorilas, A. et al. (2000) *Biochem. Biophys. Res. Commun.* 278, 349–359.
- Frankel, A. et al. (2002) *J. Biol. Chem.* 277, 3537–3543.
- Herrmann, F. et al. (2005) *J. Biol. Chem.* 280, 38005–38010.

F—Flow cytometry E—ELISA D—DELFIATM

Z—zebra fish B—bovine All—all species expected

## Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

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.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.