Jumonji Family Antibody Sampler Kit

#8340 Store at -20°C

☑ 1 Kit
(7 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev.06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
JARID1A (D28B10) XP® Rabbit mAb	3876	20 µl	200 kDa	Rabbit IgG
JARID1B Antibody	3273	20 µl	180 kDa	Rabbit IgG
JARID1C (D29B9) Rabbit mAb	5361	20 µl	180 kDa	Rabbit IgG
JMJD2A (C37E5) Rabbit mAb	5328	20 µl	150 kDa	Rabbit IgG
JMJD1B (6A1-1F5) Mouse mAb	5377	20 µl	220 kDa	Mouse IgG1
JMJD2B (D7E6) Rabbit mAb	8639	20 µl	150 kDa	Rabbit IgG
PHF2 (D45A2) Rabbit mAb	3497	20 µl	150 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The Jumonji Family Antibody Sampler Kit provides an economical means of evaluating total levels of Jumonji family proteins. The kit contains enough primary antibodies to perform two western blot experiments with each primary antibody.

Background: Jumonji C (JmjC) domain-containing proteins represent the largest class of potential histone demethylase proteins (1). The JmjC domain can catalyze the demethylation of mono-, di-, and tri-methyl lysine residues via an oxidative reaction that requires iron and α-ketoglutarate (1). Based on homology, both humans and mice contain at least 30 such proteins, which can be divided into 7 separate families (1).

The JMJD2 (Jumonji domain-containing protein 2) family, also known as JHDM3 (JmjC domain-containing histone demethylation protein 3) family, contains four members: JMJD2A/JHDM3A, JMJD2B/JHDM3B, JMJD2C/JHDM3C, and JMJD2D/JHDM3D. In addition to the JmjC domain, these proteins also contain JmjN, PHD and Tudor domains, the latter of which has been shown to bind to methylated histone H3 at Lys4 and Lys9, and methylated histone H4 at Lys20 (2,3). JMJD2 proteins have been shown to demethylate di- and tri-methyl histone H3 at Lys9 and Lys36, and function as both activators and repressors of transcription (4-9). JMJD2A, JMJD2C and JMJD2D function as coactivators of the androgen receptor in prostate tumor cells (5). In contrast, JMJD2A also associates with Rb and N-CoR corepressor complexes and is necessary for transcriptional repression of target genes (6,7). JMJD2B antagonizes histone H3 Lys9 tri-methylation at pericentric heterochromatin (8). JMJD1B is a more widely expressed family member and is frequently deleted in myeloid leukemia (10).

The JARID (Jumonji/AT-rich interactive domain-containing protein) family contains four members: JARID1A (also RBP2 and RBBP2), JARID1B (also PLU-1), JARID1C (also SMCX), and JARID1D (also SMCY) (11). In addition to the JmjC domain, these proteins contain JmjN, BRIGHT, C5HC2 zinc-finger, and PHD domains, the latter of which binds to methylated histone H3 (Lys9) (11). All four JARID proteins demethylate di- and tri-methyl histone H3 Lys4; JARID1B also demethylates mono-methyl histone H3 Lys4 (12-14). JARID1A is a critical RB-interacting protein and is required for Polycomb-Repressive Complex 2 (PRC2)-mediated transcriptional repression during ES cell differentiation (15). A JARID1A-NUP98 gene fusion is associated with myeloid leukemia (16). JARID1B, which interacts with many proteins including c-Myc and HDAC4, may play a role in cell fate decisions by blocking terminal differentiation (17-19). JARID1B is over-expressed in many breast cancers and may act by repressing multiple tumor suppressor genes including BRCA1 and HOXA5 (20,21). JARID1C has been found in a complex with HDAC1, HDAC2, G9a, and REST, which binds to and represses REST target genes in non-neuronal cells (14). JARID1D is largely uncharacterized. PHF2 contains a JmjC domain, which may play a role in histone demethylation (1).

Specificity/Sensitivity: Each antibody in the Jumonji Family Antibody Sampler Kit detects endogenous levels of the respective target protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide and are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with recombinant human proteins or synthetic peptides.

Background References:

(1) Klose, R.J. et al. (2006) *Nat Rev Genet* 7, 715-27.
(2) Chen, Z. et al. (2007) *Proc Natl Acad Sci USA* 104, 10818-23.
(3) Lee, J. et al. (2008) *Nat Struct Mol Biol* 15, 109-11.
(4) Whetstine, J.R. et al. (2006) *Cell* 125, 467-81.
(5) Shin, S. and Janknecht, R. (2007) *Biochem Biophys Res Commun* 359, 742-6.
(6) Gray, S.G. et al. (2005) *J Biol Chem* 280, 28507-18.
(7) Zhang, D. et al. (2005) *Mol Cell Biol* 25, 6404-14.
(8) Fodor, B.D. et al. (2006) *Genes Dev* 20, 1557-62.
(9) Cloos, P.A. et al. (2006) *Nature* 442, 307-11.
(10) Hu, Z. et al. (2001) *Oncogene* 20, 6946-54.
(11) Benevolenskaya, E.V. (2007) *Biochem Cell Biol* 85, 435-43.
(12) Christensen, J. et al. (2007) *Cell* 128, 1063-76.
(13) Yamane, K. et al. (2007) *Mol Cell* 25, 801-12.
(14) Tahiliani, M. et al. (2007) *Nature* 447, 601-5.
(15) Pasini, D. et al. (2008) *Genes Dev* 22, 1345-55.
(16) van Zutven, L.J. et al. (2006) *Genes Chromosomes Cancer* 45, 437-46.
(17) Secombe, J. et al. (2007) *Genes Dev* 21, 537-51.
(18) Barrett, A. et al. (2007) *Int J Cancer* 121, 265-75.
(19) Dey, B.K. et al. (2008) *Mol Cell Biol* 28, 5312-27.
(20) Barrett, A. et al. (2002) *Int J Cancer* 101, 581-8.
(21) Lu, P.J. et al. (1999) *J Biol Chem* 274, 15633-45.

U.S. Patent No. 5,675,063

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **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.

#8340

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.