Store at -20°C

SET1/COMPASS Antibody Sampler Kit

#25501

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New 10/17

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Products Included	Product #	Quantity	Mol. Wt.	Isotype
SET1A (D3V9S) Rabbit mAb	61702	20 µl	300 kDa	Rabbit IgG
SET1B (D1U5D) Rabbit mAb	44922	20 µl	320 kDa	Rabbit IgG
MLL1 (D2M7U) Rabbit mAb (Amino-terminal Antigen)	14689	20 µl	300 kDa	Rabbit IgG
MLL1 (D6G8N) Rabbit mAb (Carboxy-terminal Antigen)	14197	20 µl	180 kDa	Rabbit IgG
MLL2/KMT2B (D6X2E) Rabbit mAb (Carboxy-terminal Antigen)	63735	20 µl	80 kDa	Rabbit IgG
WDR5 (D9E1I) Rabbit mAb	13105	20 µl	37 kDa	Rabbit IgG
WDR82 (D2I3B) Rabbit mAb	99715	20 µl	30 kDa	Rabbit IgG
Menin (D45B1) XP® Rabbit mAb	6891	20 µl	68 kDa	Rabbit IgG
Anti-Rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

Description: The SET1/COMPASS Antibody Sampler Kit provides an economical means of detecting SET1/COMPASS proteins using control antibodies against SET1A, SET1B, MLL1, MLL2, WDR5, WDR82, and Menin. This kit contains enough primary antibodies to perform at least two western blot experiments.

Background: The Set1 histone methyltransferase protein was first identified in yeast as part of the Set1/COMPASS histone methyltransferase complex, which methylates histone H3 on lysine 4 and functions as a transcriptional co-activator (1). While yeast contain only one known Set1 protein, mammals contain six Set1-related proteins: SET1A, SET1B, MLL1, MLL2, MLL3 and MLL4, all of which methylate histone H3 on lysine 4 (2,3). These Set1-related proteins are each found in distinct protein complexes, all of which share the common core structural subunits WDR5, RBBP5 and ASH2L (2-6). WDR82 is a core subunit specific to SET1A and SET1B complexes, while Menin is a core subunit specific to the MLL complexes (4,5,7).

Like yeast Set1, all six Set1-related mammalian proteins methylate histone H3 on lysine 4 (2-6). SET1A, SET1B, MLL1 and MLL2 mediate di- and tri-methylation of histone H3 Lys4 at gene promoters to facilitate transcription activation. MLL3 and MLL4 function primarily to mono-methylate histone H3 Lys4 at gene enhancers. MLL1 and MLL2 function as master regulators of both embryogenesis and hematopoiesis, and are required for proper expression of Hox genes (8-10). MLL1 is a large approximately 4000 amino acid protein that is cleaved by the Taspase 1 threonine endopeptidase to form N-terminal (MLL1-N) and C-terminal MLL1 (MLL1-C) fragments, both of which are subunits of the functional MLL1/COMPASS complex (11,12). MLL1 translocations are found in a large number of hematological malignancies, suggesting that Set1 histone methyltransferase complexes play a critical role in leukemogenesis (6). Like MLL1, MLL2 is also a large, approximately 2700 amino acid protein that is cleaved by the Taspase 1 threonine endopeptidase to form N-terminal (MLL2-N) and C-terminal (MLL2-C) fragments, both of which are subunits of the functional MLL2/COMPASS complex. MLL2 has also been implicated as a modulator of hematological malignancies (13). MLL3 and MLL4 proteins are not cleaved by Taspase 1.

Specificity/Sensitivity: Each antibody in the SET1/COMPASS Antibody Sampler Kit detects endogenous levels of its target protein. Cross-reactivity was not observed with other family members.

Source/Purification: Monoclonal antibodies are produced by immunizing rabbits with synthetic peptides corresponding to residues surrounding Pro383 of human SET1A, Val204 of human SET1B, Val13 of human WDR82, and Val597 of human Menin proteins, or recombinant protein specific to the amino terminus of human MLL1, carboxy terminus of human MLL1, carboxy terminus of human MLL2, and full-length human WDR5 proteins.

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.*

Background References:

(1) Miller, T. et al. (2001) *Proc Natl Acad Sci U S A* 98, 12902-7.
(2) Shilatifard, A. (2008) *Curr Opin Cell Biol* 20, 341-8.
(3) Tenney, K. and Shilatifard, A. (2005) *J Cell Biochem* 95, 429-36.
(4) Lee, J.H. and Skalnik, D.G. (2005) *J Biol Chem* 280, 41725-31.
(5) Lee, J.H. et al. (2007) *J Biol Chem* 282, 13419-28.
(6) Hughes, C.M. et al. (2004) *Mol Cell* 13, 587-97.
(7) Yokoyama, A. et al. (2004) *Mol Cell Biol* 24, 5639-49.
(8) Eissenberg, J.C. and Shilatifard, A. (2010) *Dev Biol* 339, 240-9.
(9) Smith, E. et al. (2011) *Genes Dev* 25, 661-72.
(10) Denissov, S. et al. (2014) *Development* 141, 526-37.
(11) Takeda, S. et al. (2006) *Genes Dev* 20, 2397-409.
(12) Yokoyama, A. et al. (2002) *Blood* 100, 3710-8.
(13) Chen, Y. et al. (2017) *Cancer Cell* 31, 755-770.e6.

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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.

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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.