NuRD Complex Antibody Sampler Kit

#8349 Store at -20°C

☑ 1 Kit
(7 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
CHD3 Antibody	4241	20 µl	260 kDa	Rabbit IgG
CHD4 (D8B12) Rabbit mAb	11912	20 µl	260 kDa	Rabbit IgG
HDAC1 (10E2) Mouse mAb	5356	20 µl	62 kDa	Mouse IgG1
HDAC2 (3F3) Mouse mAb	5113	20 µl	60 kDa	Mouse IgG1
MBD3 (N87) Antibody	14540	20 µl	32, 34 kDa	Rabbit IgG
MTA1 (D40D1) XP® Rabbit mAb	5647	20 µl	78–82 kDa	Rabbit IgG
RBAP46 (V415) Antibody	6882	20 µl	48 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: NuRD Complex Antibody Sampler Kit offers an economical means of detecting each target protein that composes the nucleosome remodeling and deacetylation complex (NuRD). The kit includes enough primary antibody to perform two western blot experiments with each primary antibody.

Background: The transcriptional repressor NuRD (nucleosome remodeling and histone deacetylase) complex is composed of multiple subunits, which include CHD3 (also known as Mi2-α), CHD4 (also known as Mi2-β), HDAC1, HDAC2, RBAP48, RBAP46, MTA1, MTA2, MTA3, and MBD3 (1-6). Both CHD3 and CHD4 contain two PHD (plant homeodomain) zinc finger domains that bind directly to HDAC1 and HDAC2. HDAC1 and HDAC2 are highly homologous and are involved in histone deacetylation, chromatin remodeling and transcriptional repression, and are also found together in the mSin3A and MeCP1 complexes (7-9). RBAP46 and RBAP48 bind to the histone fold region of histone H4 and are believed to target the NuRD complex to its histone substrates (10). MBD3, a member of the methyl-CpG-binding domain protein family, and the metastasis associated genes MTA1, MTA2, and MTA3 were all found to be integral components of the NuRD complex (2,3,7,11).

Specificity/Sensitivity: All antibodies contained in this kit detect endogenous levels of their 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) Tong, J.K. et al. (1998) *Nature* 395, 917-21.
(2) Xue, Y. et al. (1998) *Mol Cell* 2, 851-61.
(3) Zhang, Y. et al. (1998) *Cell* 95, 279-89.
(4) Bowen, N.J. et al. (2004) *Biochim Biophys Acta* 1677, 52-7.
(5) Jones, P.L. et al. (1998) *Nat Genet* 19, 187-91.
(6) Fujita, N. et al. (2003) *Cell* 113, 207-19.
(7) Zhang, Y. et al. (1999) *Genes Dev* 13, 1924-35.
(8) Ng, H.H. et al. (1999) *Nat Genet* 23, 58-61.
(9) Zhang, Y. et al. (1997) *Cell* 89, 357-64.
(10) Verreault, A. et al. (1998) *Curr Biol* 8, 96-108.
(11) Wade, P.A. et al. (1999) *Nat Genet* 23, 62-6.

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.

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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.

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