

Chromodomain Helicase DNA-Binding (CHD) Family Antibody Sampler Kit



Cell Signaling
TECHNOLOGY®

✓ 1 Kit
(6 x 40 µl)

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
CHD1 (D8C2) Rabbit mAb	4351	40 µl	220 kDa	Rabbit IgG
CHD2 Antibody	4170	40 µl	260 kDa	Rabbit IgG
CHD3 Antibody	4241	40 µl	260 kDa	Rabbit IgG
CHD4 (D8B12) Rabbit mAb	11912	40 µl	260 kDa	Rabbit IgG
CHD7 (D3F5) Rabbit mAb	6505	40 µl	336 kDa	Rabbit IgG
CHD8 (D3C1) Rabbit mAb	11891	40 µl	290 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 Chromodomain Helicase DNA-Binding (CHD) Family Antibody Sampler Kit provides an economical means to investigate the CHD family members associated with the histone deacetylase NuRD complex. This kit contains enough primary antibody to perform four western blots per primary antibody.

Background: Chromodomain-helicase-DNA-binding domain (CHD) proteins have been identified in a variety of organisms (1,2). This family of nine proteins is divided into three separate subfamilies: subfamily I (CHD1 and CHD2), subfamily II (CHD3 and CHD4), and subfamily III (CHD5, CHD6, CHD7, CHD8, and CHD9). All CHD proteins contain two tandem amino-terminal chromodomains, a SWI/SNF-related ATPase domain, and a carboxy-terminal DNA-binding domain (1,2). The chromodomains facilitate binding to methylated lysine residues of histone proteins and confer interactions with specific regions of chromatin. The SWI/SNF-related ATPase domain utilizes energy from ATP hydrolysis to modify chromatin structure. CHD proteins are often found in large, multiprotein complexes with their transcriptional activation or repression activity governed by other proteins within the complex. CHD3 (also known as Mi2-α) and CHD4 (also known as Mi2-β) are central components of the nucleosome remodeling and histone deacetylase (NuRD) transcriptional repressor complex, which also contains HDAC1, HDAC2, RBAP48, RBAP46, MTA1, MTA2, MTA3, and MBD3 (3-8). Both CHD3 and CHD4 contain two plant homeodomain (PHD) zinc finger domains that bind directly to HDAC1 and HDAC2.

Specificity/Sensitivity: Each antibody in the Chromodomain Helicase DNA-Binding (CHD) Family Antibody Sampler Kit recognizes total endogenous levels of the specific target protein. CHD8 (D3C1) Rabbit mAb also cross-reacts with a protein of unknown origin at 140 kDa.

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:

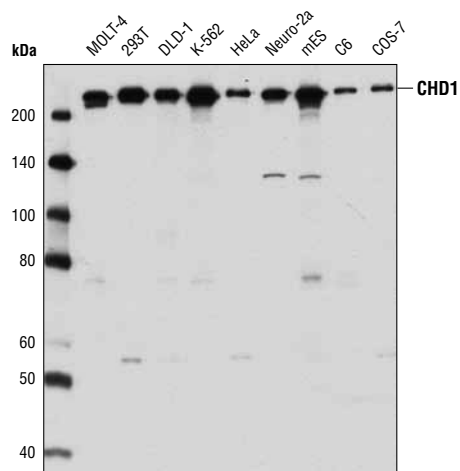
- (1) Hall, J.A. and Georgel, P.T. (2007) *Biochem Cell Biol* 85, 463-76.
- (2) Marfella, C.G. and Imbalzano, A.N. (2007) *Mutat Res* 618, 30-40.
- (3) Tong, J.K. et al. (1998) *Nature* 395, 917-21.
- (4) Xue, Y. et al. (1998) *Mol Cell* 2, 851-61.
- (5) Zhang, Y. et al. (1998) *Cell* 95, 279-89.
- (6) Bowen, N.J. et al. (2004) *Biochim Biophys Acta* 1677, 52-7.
- (7) Jones, P.L. et al. (1998) *Nat Genet* 19, 187-91.
- (8) Fujita, N. et al. (2003) *Cell* 113, 207-19.

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.

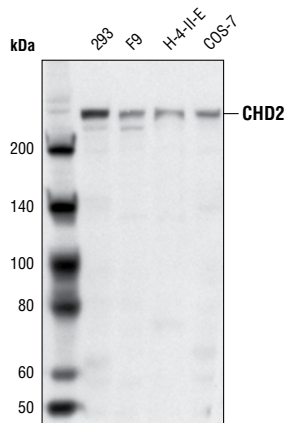
Recommended Antibody Dilutions:

Western blotting 1:1000

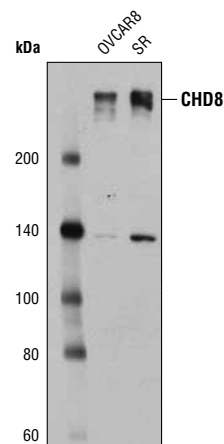
Please visit www.cellsignal.com for a complete listing of recommended companion products.



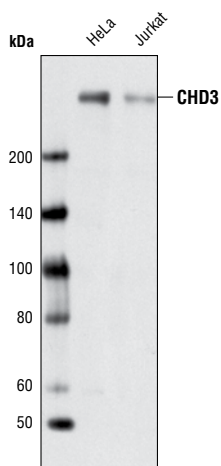
Western blot analysis of extracts from various cell lines using **CHD1 (D8C2) Rabbit mAb #4351**.



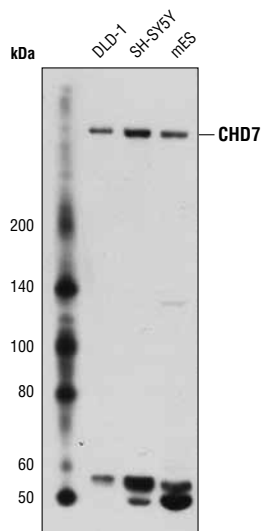
Western blot analysis of extracts from various cell lines using **CHD2 Antibody #4170**.



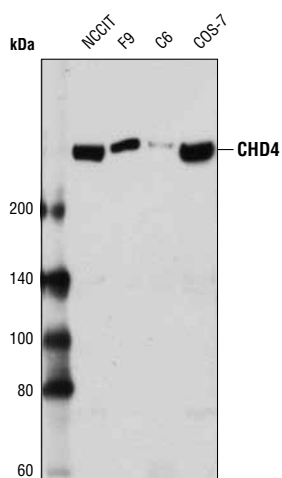
Western blot analysis of extracts from OVCAR8 and SR cells using **CHD8 (D3C1) Rabbit mAb #11891**.



Western blot analysis of extracts from HeLa and Jurkat cells using **CHD3 Antibody #4241**.



Western blot analysis of extracts from DLD-1, SH-SY5Y and mES cells using **CHD7 (D3F5) Rabbit mAb #6505**.



Western blot analysis of extracts from various cell lines using **CHD4 (D8B12) Rabbit mAb #11912**.

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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 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 dH₂O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 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 dH₂O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- 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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- 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.
- Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

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

II. Primary Antibody Incubation

- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- 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.
- 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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