

# Histone Deacetylase (HDAC) Antibody Sampler Kit

1 Kit  
 (6 x 20 µl)



**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
HDAC1 (10E2) Mouse mAb	5356	20 µl	62 kDa	Mouse IgG1
HDAC2 (3F3) Mouse mAb	5113	20 µl	60 kDa	Mouse IgG1
HDAC3 (7G6C5) Mouse mAb	3949	20 µl	49 kDa	Mouse IgG2a
HDAC4 (D15C3) Rabbit mAb	7628	20 µl	140 kDa	Rabbit IgG
Phospho-HDAC4 (Ser246)/HDAC5 (Ser259)/HDAC7 (Ser155) (D27B5) Rabbit mAb	3443	20 µl	140, 124, 120 kDa	Rabbit IgG
HDAC6 (D2E5) Rabbit mAb	7558	20 µl	160 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](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The Histone Deacetylase (HDAC) Antibody Sampler Kit provides an economical means to evaluate the endogenous levels of HDACs. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

**Background:** Acetylation of the histone tail causes chromatin to adopt an "open" conformation, allowing increased accessibility of transcription factors to DNA. The identification of histone acetyltransferases (HATs) and their large multiprotein complexes has yielded important insights into how these enzymes regulate transcription (1,2). HAT complexes interact with sequence-specific activator proteins to target specific genes. In addition to histones, HATs can acetylate nonhistone proteins, suggesting multiple roles for these enzymes (3). In contrast, histone deacetylation promotes a "closed" chromatin conformation and typically leads to repression of gene activity (4). Mammalian histone deacetylases can be divided into three classes on the basis of their similarity to various yeast deacetylases (5). Class

I proteins (HDACs 1, 2, 3, and 8) are related to the yeast Rpd3-like proteins, those in class II (HDACs 4, 5, 6, 7, 9, and 10) are related to yeast Hda1-like proteins, and class III proteins are related to the yeast protein Sir2. Inhibitors of HDAC activity are now being explored as potential therapeutic cancer agents (6,7).

**Specificity/Sensitivity:** The Histone Deacetylase (HDAC) Antibody Sampler Kit provides a fast and economical means to evaluate the endogenous levels of HDACs. The kit contains enough primary and secondary antibodies to perform four mini-blot experiments. Each antibody in the Histone Deacetylase (HDAC) Antibody Sampler Kit detects endogenous levels of its target.

**Source/Purification:** Monoclonal antibodies are produced by immunizing animals with a recombinant protein specific to the carboxy terminus of human HDAC6, the amino terminus of human HDAC4 protein, synthetic peptides to the carboxy-terminal residues of human HDAC1 and

**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 antibodies.*

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

**Please visit [www.cellsignal.com](http://www.cellsignal.com) for validation data and a complete listing of recommended companion products.**

HDAC2 proteins, or synthetic phosphopeptide corresponding to Ser155 of human HDAC7 protein. HDAC3 (7G6C5) monoclonal antibody is produced by immunizing animals with recombinant human HDAC3 protein.

**Background References:**

- (1) Marmorstein, R. (2001) *Cell Mol Life Sci* 58, 693-703.
- (2) Gregory, P.D. et al. (2001) *Exp Cell Res* 265, 195-202.
- (3) Liu, Y. et al. (2000) *Mol Cell Biol* 20, 5540-53.
- (4) Cress, W.D. and Seto, E. (2000) *J Cell Physiol* 184, 1-16.
- (5) Gray, S.G. and Ekström, T.J. (2001) *Exp Cell Res* 262, 75-83.
- (6) Thiagalingam, S. et al. (2003) *Ann. N.Y. Acad. Sci.* 983, 84-100.
- (7) Vigushin, D.M. and Coombes, R.C. (2004) *Curr. Cancer Drug Targets* 4, 205-218.

# 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 dH<sub>2</sub>O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH<sub>2</sub>O, 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 dH<sub>2</sub>O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH<sub>2</sub>O, 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 dH<sub>2</sub>O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH<sub>2</sub>O, 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 cm<sup>2</sup>) 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.