Store at -20°C

#89985

Huntingtin Interaction Antibody Sampler Kit

1 Kit (6 x 20 µl)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

Rev 09/19/17

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
PPARγ (C26H12) Rabbit mAb	2435	20 µl	53, 57 kDa	Rabbit IgG
CtBP1 (D2D6) Rabbit mAb	8684	20 µl	47 kDa	Rabbit IgG
p53 (7F5) Rabbit mAb	2527	20 µl	53 kDa	Rabbit IgG
SUMO-1 (C9H1) Rabbit mAb	4940	20 µl		Rabbit IgG
NF-κ-B1 p105/p50 (D4P4D) Rabbit mAb	13586	20 µl	50 kDa Active form. 120 kDa Precursor	Rabbit IgG
ACF1 Rabbit Ab	6255	20 µl	203 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.

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

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Description: The Huntingtin Interaction Antibody Sampler Kit provides an economical means of detecting transcription-related proteins that interact with Huntingtin (Htt). This kit contains enough antibody to perform two western blot experiments per primary antibody.

Background: Peroxisome proliferator-activated receptor gamma (PPARG) is a member of the ligand-activated nuclear receptor superfamily and functions as a transcriptional activator (1). Besides its role in mediating adipogenesis and lipid metabolism (2), PPARγ also modulates insulin sensitivity, cell proliferation and inflammation (3). CtBP1 is able to regulate gene activity through its intrinsic dehydrogenase activity (4,5) and by interacting with Polycomb Group (PcG) proteins during development (6). Along with its homologue, CtBP2, it acts as a transcriptional corepressor of zinc-finger homeodomain factor deltaEF1 to regulate a wide range of cellular processes through transrepression mechanisms (7). The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis (8). DNA damage induces phosphorylation of p53 at Ser15 and Ser20 and leads to a reduced interaction between p53 and its negative regulator, the oncoprotein MDM2 (9). MDM2 inhibits p53 accumulation by targeting it for ubiquitination and proteasomal degradation (10,11). Phosphorylation impairs the ability of MDM2 to bind p53, promoting both the accumulation and activation of p53 in response to DNA damage (9,12). Acetylation appears to play a positive role in the accumulation of p53 protein in stress response (13). Deacetylation of p53 occurs through interaction with the SIRT1 protein, a deacetylase that may be involved in cellular aging and the DNA damage response (14). Small ubiquitin-related modifier 1, 2 and 3 (SUMO-1, -2 and -3) are members of the ubiquitin-like protein family (15). The covalent attachment of the SUMO-1, -2 or -3 (SUMOylation) to target proteins is analogous to ubiquitination. Ubiquitin and the individual SUMO family members are all targeted to different proteins with diverse biological functions. Ubiquitin predominantly regulates degradation of its target (1). In contrast, SUMO-1 is conjugated to RanGAP, PML, p53 and IkB-alpha to regulate nuclear trafficking, formation of subnuclear structures, regulation of transcriptional activity and protein stability (16-20). Transcription factors of the nuclear factor kappaB (NF-kB)/Rel family play a pivotal role in inflammatory and immune responses (21, 22). In unstimulated cells, NF-kB is sequestered in the cytoplasm by IkB inhibitory proteins (23-25). NF-kB-activating agents can induce the phosphorylation of IkB proteins, targeting them for rapid degradation through the ubiquitin-proteasome pathway and releasing NF-kB to enter the nucleus where it regulates gene expression (26-28). ACF1 (BAZ1A) has distinct roles in development (29), regulation of chromatin structure (30), and DNA damage response (31, 32). Different developmental stages dictate the expression of ACF1 in Drosophila, and alterations in ACF1 expression during Drosophila development leads to deviation from normal chromatin organization (29).

Specificity/Sensitivity: Unless otherwise indicated, each antibody will recognize endogenous levels of total target protein. SUMO-1 (C9H1) Rabbit mAb detects recombinant SUMO-1 and endogenous levels of sumoylated proteins (e.g. SUMO-1-RanGAP at 90 kD). SUMO-1 (C9H1) Rabbit mAb does not detect recombinant SUMO-2 or SUMO-3. ACF1 Antibody recognizes endogenous levels of total ACF1 protein (isoforms 1 and 2).

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the residues surrounding Asp69 of human PPARγ, amino terminus of the human CtBP1 protein, full-length human p53 fusion protein, amino terminus of human SUMO-1, or residues surrounding Ile415 of mouse NF-kB1 P105/p50 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponds to the residues surrounding Met864 of human ACF1 protein. Antibodies are purified by protein A and peptide affinity chromatography

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

Thank you for your recent purchase. If you would like to provide a review visit cellsignal.com/comments.

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.

Background References:

(1) Tontonoz, P. et al. (1995) *Curr Opin Genet Dev* 5, 571-6.

(2) Rosen, E.D. et al. (1999) *Mol Cell* 4, 611-7.

(3) Murphy, G.J. and Holder, J.C. (2000) *Trends Pharmacol Sci* 21, 469-74.

(4) Balasubramanian, P. et al. (2003) *FEBS Lett* 537, 157-60.

(5) Kumar, V. et al. (2002) *Mol Cell* 10, 857-69.

(6) Sewalt, R.G. et al. (1999) *Mol Cell Biol* 19, 777-87.

(7) Furusawa, T. et al. (1999) *Mol Cell Biol* 19, 8581-90.

(8) Levine, A.J. (1997) *Cell* 88, 323-31.

(9) Shieh, S.Y. et al. (1997) *Cell* 91, 325-34.

(10) Chehab, N.H. et al. (1999) *Proc Natl Acad Sci USA* 96, 13777-82.

(11) Honda, R. et al. (1997) *FEBS Lett* 420, 25-7.

(12) Tibbetts, R.S. et al. (1999) *Genes Dev* 13, 152-7.

(13) Ito, A. et al. (2001) *EMBO J* 20, 1331-40.

(14) Solomon, J.M. et al. (2006) *Mol Cell Biol* 26, 28-38.

(15) Schwartz, D.C. and Hochstrasser, M. (2003) *Trends Biochem Sci* 28, 321-8.

(16) Matunis, M.J. et al. (1996) *J Cell Biol* 135, 1457-70.

(17) Duprez, E. et al. (1999) *J Cell Sci* 112 (Pt 3), 381-93.

(18) Gostissa, M. et al. (1999) *EMBO J* 18, 6462-71.

(19) Rodriguez, M.S. et al. (1999) *EMBO J* 18, 6455-61.

(20) Desterro, J.M. et al. (1998) *Mol Cell* 2, 233-9.

(21) Baeuerle, P.A. and Henkel, T. (1994) *Annu Rev Immunol* 12, 141-79.

(22) Baeuerle, P.A. and Baltimore, D. (1996) *Cell* 87, 13-20.

(23) Haskill, S. et al. (1991) *Cell* 65, 1281-9.

(24) Thompson, J.E. et al. (1995) *Cell* 80, 573-82.

(25) Whiteside, S.T. et al. (1997) *EMBO J* 16, 1413-26.

(26) Traenckner, E.B. et al. (1995) *EMBO J* 14, 2876-83.

(27) Scherer, D.C. et al. (1995) *Proc Natl Acad Sci USA* 92, 11259-63.

(28) Chen, Z.J. et al. (1996) *Cell* 84, 853-62.

(29) Chioda, M. et al. (2010) *Development* 137, 3513-22.

(30) Ho, L. and Crabtree, G.R. (2010) *Nature* 463, 474-84.

(31) Sànchez-Molina, S. et al. (2011) *Nucleic Acids Res* 39, 8445-56.

(32) Lan, L. et al. (2010) *Mol Cell* 40, 976-87.

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

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.

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.

Orders: 877-616-2355 | orders@cellsignal.com | **Support:** +1-978-867-2388 | www.cellsignal.com/support