Store at -20°C

#8353

DUB Antibody Sampler Kit

☑ 1 Kit
(9 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
CYLD (D1A10) Rabbit mAb	8462	20 µl	108 kDa	Rabbit IgG
Phospho-CYLD (Ser418) Antibody	4500	20 µl	108 kDa	Rabbit IgG
STAMBP Antibody	5245	20 µl	50 kDa	Rabbit IgG
A20/TNFAIP3 (D13H3) Rabbit mAb	5630	20 µl	82 kDa	Rabbit IgG
UCHL1 (D3T2E) XP® Rabbit mAb	13179	20 µl	27 kDa	Rabbit IgG
UCHL3 (D25E6) Rabbit mAb	8141	20 µl	27 kDa	Rabbit IgG
HAUSP (D17C6) XP® Rabbit mAb	4833	20 µl	135, 140 kDa	Rabbit IgG
USP10 (D7A5) Rabbit mAb	8501	20 µl	110 kDa	Rabbit IgG
USP9X Antibody	5751	20 µl	270 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

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

Description: The DUB Antibody Sampler Kit offers an economical means of evaluating the presence and status of selected DUB enzymes. This kit includes enough primary antibody to perform two western blot experiments for each primary antibody.

Background: Ubiquitinating enzymes (UBEs) catalyze protein ubiquitination, a reversible process countered by deubiquitinating enzyme (DUB) action (1,2). CYLD deubiquitinase regulates inflammation and cell proliferation by down regulating NF-κB signaling through removal of ubiquitin chains from several NF-κB pathway proteins (3,4). Phosphorylation at Ser418 decreases CYLD deubiquitinase activity and is important for IKKε-driven transformation (5). STAM-binding protein (STAMBP or AMSH) is an endosomal DUB that preferentially displays ubiquitin isopeptidase activity toward K63-linked chains (6,7). The amino-terminus of A20 contains deubiquitinating activity for Lys63 branches, such as those found in TRAF6 and RIP, while the carboxyl-terminus contains ubiquitin ligase activity for Lys48 branches of the same substrates and leads to their degradation (8). Both enzymes have been implicated in neurodegenerative diseases (9-11) and play a role in the regulation of neuronal development and spermatogenesis (10,13,14). UCHL1 binds monoubiquitin and UCHL3 shows affinity for both ubiquitin and NEDD8, a ubiquitin-like molecule (11,12). HAUSP can bind and deubiquitinate the p53 transcription factor and an associated regulator protein Mdm2, thereby stabilizing both proteins (15,16). HAUSP also modifies other ubiquitinated proteins such as members of the FoxO family of forkhead transcription factors and the mitotic stress checkpoint protein CHFR (17,18). USP10 appears to be regulated through both protein-protein interactions and phosphorylation. Interaction of USP10 with Ras-GAP SH3 domain binding protein (G3BP) inhibits its ability to disassemble ubiquitin chains (19). ATM-mediated phosphorylation at Thr42 and Ser337 stabilizes USP10, promoting redistribution from the cytoplasm to the nucleus, where it functions in p53 deubiquitination, stabilization, and activation in response to genotoxic stress (20). USP9X possesses a well-conserved catalytic domain with cysteine peptidase activity, which allows for cleavage of ubiquitin and polyubiquitin conjugates. While USP9X expression has been shown to be critical for normal mammalian development (21-23), many of its substrates are only beginning to be elucidated.

Specificity/Sensitivity: CYLD (D1A10) Rabbit mAb recognizes endogenous levels of total CYLD protein. This antibody also detects an unidentified protein of approximately 28 kDa in some cell types. Phospho-CYLD (Ser418) Antibody detects endogenous levels of CYLD protein only when phosphorylated at Ser418. USP9X Antibody recognizes endogenous levels of total USP9X protein and may also cross-react with USP9Y. All other antibodies in this kit detect endogenous levels of total target protein.

Source/Purification: Phospho-CYLD (Ser418) Antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser418 of human CYLD protein. The remaining polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly194 of human STAMBP protein or Phe2130 of human USP9X protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Lys37 of human CYLD protein, the amino terminus of human UCHL3 protein, the amino terminus of human USP10 protein, or to the carboxy terminus of human HAUSP protein, or the carboxy terminus of human UCHL1 protein, or a recombinant protein specific to the amino terminus of human A20/TNFAIP3 protein. A20/TNFAIP3 (D13H3) Rabbit mAb was prepared in collaboration with Dr. Marc Schmidt-Supprian, Max Planck Institute of Biochemistry.

Background References:

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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 **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.