#12894 Store at -20°C

USP Antibody Sampler Kit

☑ 1 Kit
(9 x 40 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
HAUSP (D17C6) XP® Rabbit mAb	4833	40 µl	135, 140 kDa	Rabbit IgG
USP1 (D37B4) Rabbit mAb	8033	40 µl	110 kDa	Rabbit IgG
USP2 Antibody	8036	40 µl	68 kDa	Rabbit IgG
USP8 Antibody	8728	40 µl	130 kDa	Rabbit IgG
USP9X Antibody	5751	40 µl	270 kDa	Rabbit IgG
USP10 (D7A5) Rabbit mAb	8501	40 µl	110 kDa	Rabbit IgG
USP14 (D8Q6S) Rabbit mAb	11931	40 µl	60 kDa	Rabbit IgG
USP18 (D4E7) Rabbit mAb	4813	40 µl	34, 39 kDa	Rabbit IgG
USP28 Antibody	4217	40 µl	135 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 a complete listing of recommended companion products.

Description: The USP Antibody Sampler Kit provides an economical means of detecting members of the ubiquitin-specific protease (USP) family. The kit includes enough primary antibody to perform four western blot experiments per primary antibody.

Background: Ubiquitinating enzymes (UBEs) catalyze protein ubiquitination, a reversible process countered by deubiquitinating enzyme (DUB) action (1,2). The ubiquitin-specific protease (USP) subfamily is one of five distinct groups of DUB enzymes. Ubiquitin-specific-processing protease 1 (USP1) is regulated in a cell cycle dependent manner by both transcriptional and ubiquitin-proteasomal mechanisms (3). Nuclear USP1 localizes to chromatin where it deubiquitinates monoubiquitinated FANCD2 and plays an important role in DNA damage repair and Chk1 protein stability (3,4). Ubiquitin-specific-processing protease 2 (USP2) contains C19 peptidase activity and is involved in ubiquitin recycling and disassembly of polymeric ubiquitin and ubiquitin-like protein complexes (5). USP2 is a putative oncoprotein that is highly over expressed in prostate cancer and drives tumor growth by binding and stabilizing fatty acid synthase through deubiquitination (6,7).

Herpesvirus-associated ubiquitin-specific protease (HAUSP, USP7) binds and deubiquitinates transcription factor p53 and regulator protein Mdm2, stabilizing both proteins (8,9). HAUSP modifies other ubiquitinated proteins, including FoxO family forkhead transcription factors and the mitotic stress checkpoint protein CHFR (10,11). Ubiquitin-specific protease 8 (USP8, UBPy) is a cysteine protease and growth-regulated enzyme that is essential for cell proliferation and survival (12,13). The catalytic domain of USP9X possesses cysteine peptidase activity that cleaves ubiquitin and polyubiquitin conjugates. USP9X may help stabilize adherens and tight junction molecules during epithelial cell polarization (14,15). USP10 is regulated at the posttranslational level through protein-protein interactions and phosphorylation. Interaction of USP10 with the Ras-GAP SH3 domain binding protein (G3BP) inhibits the ability of USP10 to catalyze ubiquitin chain disassembly (16). ATM-mediated phosphorylation of USP10 at Thr42 and Ser337 promotes USP10 stabilization and relocation from the cytoplasm to the nucleus, where it functions in p53 deubiquitination, stabilization, and activation in response to genotoxic stress (17).

USP14 is recruited to the proteasome through association with the PSMD2 (S2/hRPN1) subunit of the 19S regulatory particle, where it may antagonize substrate degradation (18,19). USP14 trims ubiquitin residues from distal polyubiquitin chain ends, decreasing chain affinity for proteasome ubiquitin receptors and allowing for enhanced substrate stability (20,21). USP18 (UBP43) catalyzes the removal of the interferon-regulated, ubiquitin-like protein ISG15 from conjugated proteins (22). Removal of ISG15 from target proteins maintains a critical balance of cellular ISG15-conjugated proteins, which is important for normal development and brain function (23,24). USP28 can bind, deubiquitinate and stabilize several DNA-damage pathway proteins, including p53BP1 and Chk2 (25). USP28 plays an important role in Myc-related signaling as it catalyzes Myc deubiquitination and promotes Myc stabilization, which contributes to tumor-cell growth (26).

Specificity/Sensitivity: Each antibody in the USP Antibody Sampler Kit recognizes endogenous levels of its respective target protein, except for USP28, which detects transfected levels of its target protein. USP2 Antibody cross-reacts with all known USP2 splice variants but does not cross-react with USP21. USP9X Antibody may also cross-react with USP9Y. Based on sequence alignment, USP14 Antibody is predicted to cross-react with both isoform a and isoform b of USP14. The doublet band detected by western blot for USP18 (D4E7) Rabbit mAb represents full-length (39 kDa) and amino-terminal deleted derivative of USP18 (31).

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the carboxy terminus of human HAUSP protein, residues surrounding Leu768 of human USP1 protein, the amino terminus of human USP10 protein, residues near the carboxy terminus of human USP14 protein, or residues surrounding Pro45 of human USP18 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu387 of human USP2 protein, Pro320 of human USP8 protein, Phe2137 of human USP9X protein, or residues surrounding Ala11 of human USP28. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

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.

Background References:

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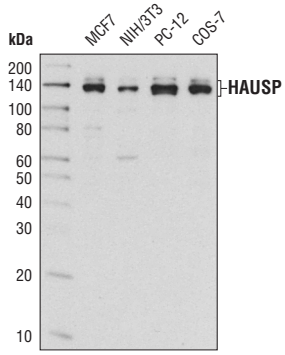
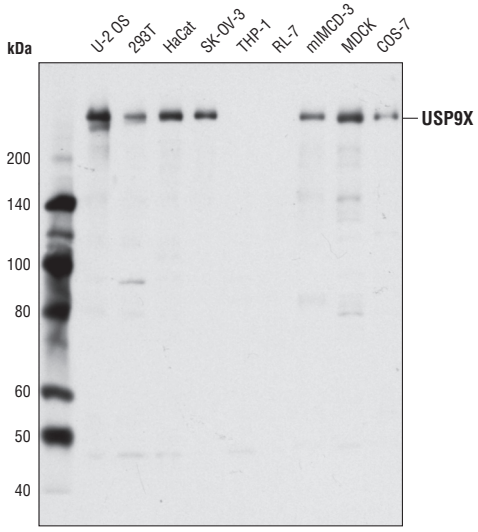
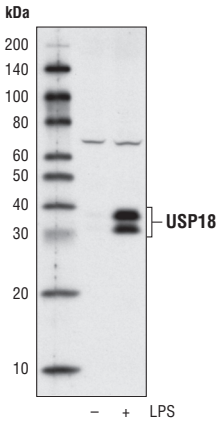


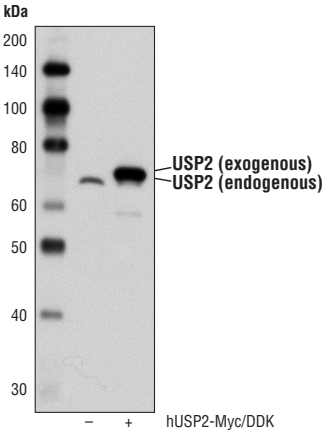
*Western blot analysis of extracts from various cell lines using **HAUSP (D17C6) XP® Rabbit mAb #4833**.*



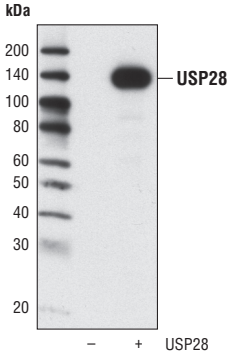
*Western blot analysis of extracts from various cell lines using **USP9X Antibody #5751**.*



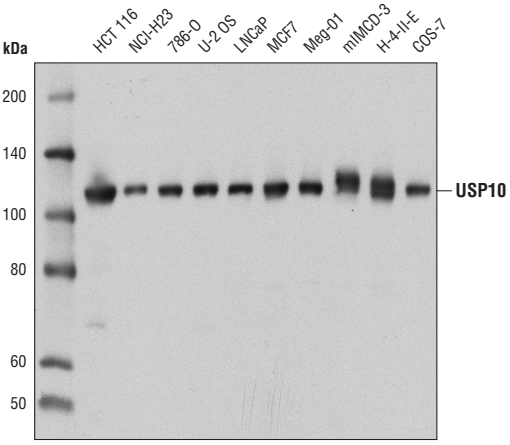
*Western blot analysis of extracts from THP-1 cells, untreated (-) or LPS-treated (1 µg/ml, 24 hr; +), using **USP18 (D4E7) Rabbit mAb #4813**.*



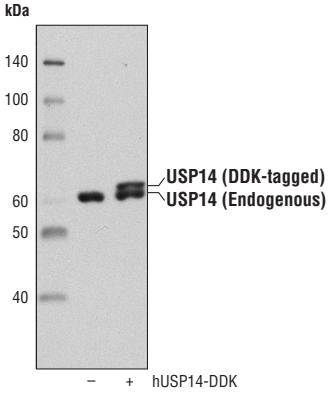
*Western blot analysis of extracts from COS-7 cells, mock transfected (-) or transfected with a Myc/DDK-tagged cDNA expression construct encoding full-length transcript variant 1 of human USP2 (hUSP2-Myc/DDK; +), using **USP2 Antibody #8036**.*



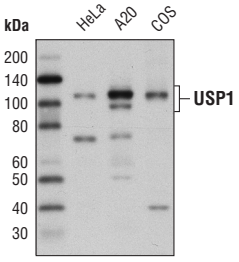
*Western blot analysis of extracts from COS cells, untransfected (-) or transfected with mouse USP28 (+), using **USP28 Antibody #4217**.*



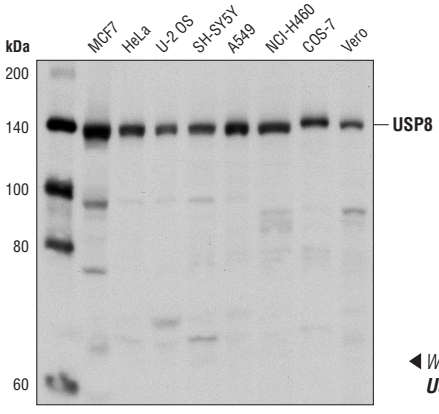
*Western blot analysis of extracts from various cell lines using **USP10 (D7A5) Rabbit mAb #8501**.*



*Western blot analysis of extracts from 293T cells, mock transfected (-) or transfected with a construct expressing DDK-tagged full-length human USP14 isoform a (hUSP14-DDK; +), using **USP14 (D8Q6S) Rabbit mAb #11931**.*



*Western blot analysis of extracts from HeLa, A20 and COS cells using **USP1 (D37B4) Rabbit mAb #8033**.*



◀ *Western blot analysis of extracts from various cell lines using **USP8 Antibody #8728**.*

#12894

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.