Ubiquitin Activation (E1, E2 Enzymes) Antibody Sampler Kit

1 Kit 
(6 x 40 µl)

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

The Ubiquitin Activation (E1, E2 Enzymes) Antibody Sampler Kit provides an economical means to study ubiquitin activation and conjugation. This kit contains enough primary antibody to perform four western blots per primary.

Background: Ubiquitin is a conserved polypeptide unit that plays an important role in the ubiquitin-proteasome pathway. Ubiquitin can be covalently linked to many cellular proteins for degradation by the 26S proteasome. Three components are involved in the target protein-ubiquitin conjugation process. Ubiquitin is first activated by forming a thioester complex with the ubiquitin-activating enzyme (UBE1 or E1). The activated ubiquitin is subsequently transferred to the ubiquitin-carrier protein (conjugating enzyme) E2, and then from E2 to ubiquitin ligase E3 for final delivery to the epsilon-NH2 of the target protein lysine residue (1-3).

The ubiquitin-proteasome pathway has been implicated in a wide range of normal biological processes and in disease-related abnormalities. Several proteins such as IκB, p53, PCNA, the IκB kinase protein NEMO, and the apoptosis inhibitor p27 for proteolysis (8). UBC3, the mammalian ortholog of yeast cdc34, and UBC3B, a UBC3 family member, are E2 components that also include UbcH5A and UbcH5C. UbcH5C is a member of the UbcH5 family that also includes UbcH5A and UbcH5B. UbcH5C catalyzes the degradation of -catenin (9). UbcH5C forms a heterodimer with MMS2 or Uev1A to exert its E2 ligase function. The UBC3 and UBC3B heterodimers catalyze different modes of target protein ubiquitination to mediate various signaling pathways (23-25) including DNA damage and recombination, p53 and check point control, cell cycle (26-30), immunoreceptor signaling (31,32), and endocytosis (33).

Specificity/Sensitivity: Ubiquitin Antibody recognizes endogenous levels of ubiquitin, polyubiquitin, and ubiquitinated proteins. This antibody may cross-react with recombinant NEDD8. UBE1a Antibody recognizes endogenous levels of total UBE1a protein. UBC3 Antibody recognizes endogenous levels of total UBC3 protein. UBC3B Antibody recognizes endogenous levels of total UBC3B protein. UBC3 Antibody recognizes endogenous levels of total UBE1a protein. UBE2L3 (D5G1) Rabbit mAb recognizes endogenous levels of total UBE2L3 protein. UBE2N (D2A1) Rabbit mAb recognizes endogenous levels of total UBE2N protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of the human ubiquitin protein, the amino terminus of human UBE1 protein, or the sequence of human UBE3 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated (-) or treated with the proteasome inhibitor MG132 (+), using Ubiquitin Antibody #3933.

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.

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry CLIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA-Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mm—monkey Mm—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

Dg—dog Pq—pig Sc—S. cerevisiae All—all species expected

Species enclosed in parentheses are predicted to react based on 100% homology.

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### Products Included

<table>
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<tr>
<th>Product</th>
<th>Product #</th>
<th>Quantity</th>
<th>Mol. Wt.</th>
<th>Isotype</th>
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<td>7074</td>
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See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated (-) or treated with the proteasome inhibitor MG132 (+), using Ubiquitin Antibody #3933.

Western blot analysis of extracts from K562 and SK-N-MC cells using UBE1a Antibody #4890.
Western blot analysis of extracts from various cell lines using **UBC3 Antibody #4997**.

Western blot analysis of extracts from Jurkat, CAD, and C6 cells using **UBC3 Antibody #4997**.

Western blot analysis of extracts from various cell lines using **UBE2L3 (D5G1) Rabbit mAb #8721**.

Western blot analysis of extracts from various cell lines using **UBE2L3 (D5G1) Rabbit mAb #8721**.

Western blot analysis of extracts from various cell lines using **UBE2N (D2A1) Rabbit mAb #6999**.

**Background References:**

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 dH2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 10 ml 10X to 900 ml dH2O, 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 3X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH2O.
4. **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.
5. **10X Tris-Glycine Transfer Buffer:** (#12339) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBS):** (#9998) 1X TBST: add 100 ml 10X TBST to 900 ml dH2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% 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 TBS 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 TBS 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)
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 to 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 determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.