

Protein Folding and Stability Antibody Sampler Kit

1 Kit
 (8 x 40 µl)



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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Skp1 Antibody	2156	40 µl	19 kDa	Rabbit IgG
Skp2 Antibody	4358	40 µl	48 kDa	Rabbit IgG
ISG15 (22D2) Rabbit mAb	2758	40 µl	15 kDa	Rabbit IgG
NEDD8 (19E3) Rabbit mAb	2754	40 µl	9 kDa	Rabbit IgG
Ubiquitin (P4D1) Mouse mAb	3936	40 µl		Mouse IgG1 κ
UBC3 Antibody	4997	40 µl	32 kDa	Rabbit IgG
SUMO-1 Antibody	4930	40 µl		Rabbit IgG
SUMO-2/3 (18H8) Rabbit mAb	4971	40 µl		Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Goat

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

Description: This sampler kit provides an economical means to investigate protein folding and stability. The kit contains primary and secondary antibodies to perform four Western blots with each antibody.

Background: The small regulatory protein ubiquitin is often covalently linked to many cellular proteins, labeling these targeted proteins for proteasome-mediated degradation. Ubiquitin is first activated by forming a thiolester complex with the E1 activation component. Activated ubiquitin is subsequently transferred to the ubiquitin-carrier protein E2, and then to an E3 ubiquitin ligase for final delivery to the ε-NH2 of the target protein lysine residue (1). The ubiquitin-proteasome pathway has been implicated in a wide range of both normal biological processes and diseases (2,3).

The ubiquitin-like protein family contains three small ubiquitin-related modifier proteins (SUMO-1, -2 and -3), neural precursor cell-expressed developmentally down-regulated protein 8 (NEDD8) and interferon-stimulated 15 kDa protein (ISG15) (4-6). Their covalent attachment to target proteins is a reversible, multi-step process that is analogous to protein ubiquitination. Mature molecules are linked to the activating enzyme E1, conjugated to E2 and ligated to the target proteins by E3 (7-10). Ubiquitin is the predominant regulator for the degradation of a wide range of target proteins (8) while SUMO, NEDD8 and ISG15 modify a limited set of substrates to regulate various other biological processes (4, 11-18).

During ubiquitination, the combinatorial interaction of different E2 and E3 proteins produces variable substrate specificity (4). UBC3 and UBC3B are E2 ubiquitin-carrier proteins (19, 20). The SCF (Skp1/CUL1/F-box) E3 ubiquitin ligase protein complex is composed of three protein components, including the S phase kinase associated protein 1 (Skp1), Cullin homolog 1 (CUL1) and the Skp2 F-box protein (21-23).

Specificity/Sensitivity: Skp1 Antibody detects endogenous levels of total Skp1 protein. Skp2 Antibody detects endogenous levels of total Skp2 protein (α, β and γ isoforms) and does not cross-react with other SKP proteins. ISG15 (22D2) Rabbit mAb detects endogenous levels of the uncleaved precursor form of ISG15 protein. This antibody does not recognize the activated (cleaved) or conjugated forms of ISG15. The antibody does not cross-react with other ubiquitin family members, including ubiquitin, SUMO-1, SUMO-2, SUMO-3 and NEDD8. NEDD8 (19E3) Rabbit mAb detects endogenous levels of both free and conjugated NEDD8 protein. The antibody does not cross-react with other ubiquitin family members, including ubiquitin, SUMO-1, SUMO-2, SUMO-3 and ISG15. Ubiquitin (P4D1) Mouse mAb detects ubiquitin, polyubiquitin and ubiquitinated proteins. UBC3 Antibody detects endogenous levels of total UBC3 and UBC3B protein. SUMO-1 Antibody detects recombinant SUMO-1 and endogenous levels of sumoylated proteins (e.g. SUMO-1-RanGAP, 90kD). SUMO-2/3 Antibody detects endogenous levels of SUMO-2/3, but does not cross-react with recombinant SUMO-1.

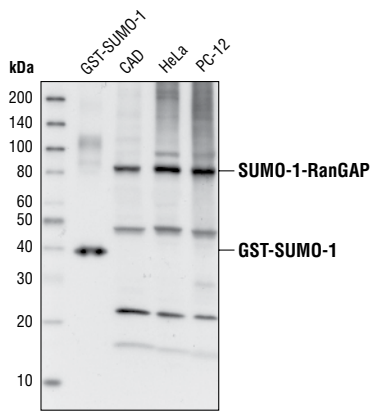
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

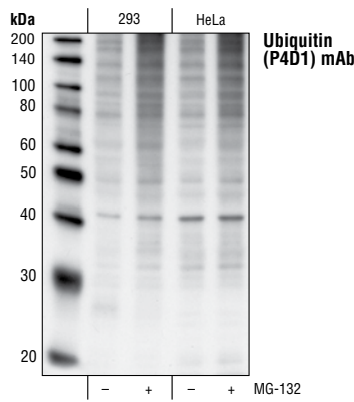
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to human SUMO-1 (#4930), UBC3 (#4997), Skp2 (#4358), and Skp1 (#2156). Polyclonal antibodies are purified by protein A and peptide affinity chromatography. SUMO-2/3 (18H8) Rabbit mAb (#4971) is produced by immunizing rabbits with a synthetic peptide from the amino terminus of human SUMO-3. Ubiquitin (P4D1) Mouse monoclonal antibody (#3936) is produced by immunizing mice with 1-76 full-length ubiquitin of bovine origin. ISG15 (22D2) Rabbit mAb is produced by immunizing animals with a synthetic peptide corresponding to amino acids at the amino terminus of human ISG15 protein. NEDD8 (19E3) Rabbit mAb is produced by immunizing animals with a synthetic peptide corresponding to amino acids at the amino terminus of human NEDD8 protein.

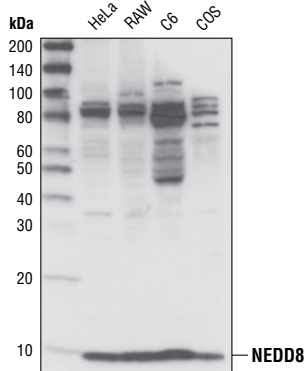
Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.



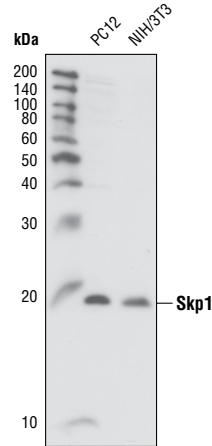
Western blot analysis of recombinant GST-SUMO-1 protein (38 kDa) and extracts from CAD, HeLa and PC-12 cells using **SUMO-1 Antibody #4930**.



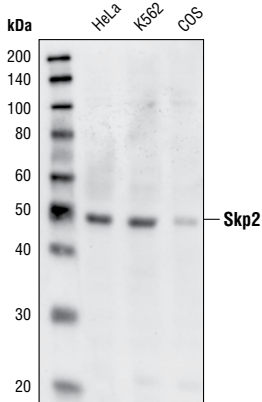
Western blot analysis of extracts from 293 and HeLa cells, untreated or treated with 50 μ M MG-132 (a 26S proteasome inhibitor) for 90 minutes, using **Ubiquitin (P4D1) Mouse mAb #3936**.



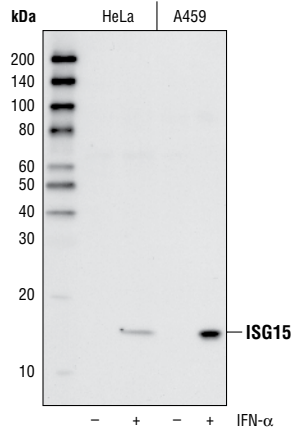
Western blot analysis of lysates from HeLa, RAW, C6 and COS cells, using **NEDD8 (19E3) Rabbit mAb #2754**.



Western blot analysis of extracts from PC12 and NIH/3T3 cells using **Skp1 Antibody #2156**.



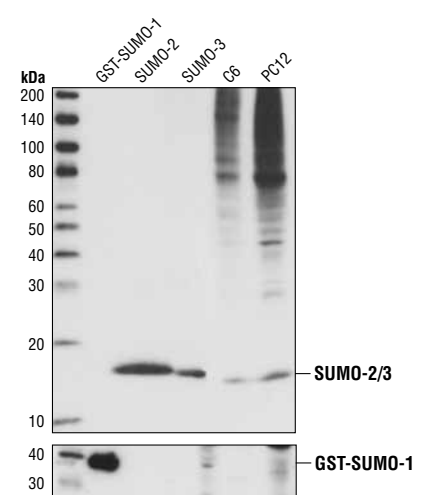
Western blot analysis of extracts from HeLa, K562 and COS cells using **Skp2 Antibody #4358**.



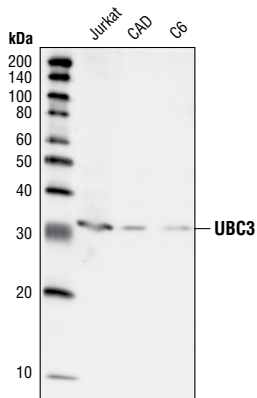
Western blot analysis of lysates from HeLa and A549 cells, untreated (-) or treated with IFN- α (1000 U/mL) (+) for 24 hours, using **ISG15 (22D2) Rabbit mAb #2758**.

Background References:

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- (3) Jesenberger, V. and Jentsch, S. (2002) *Nat Rev Mol Cell Biol* 3, 112–121.
- (4) Schwartz, D.C. and Hochstrasser, M. (2003) *Trends Biochem. Sci.* 28, 321–328.
- (5) Chiba, T. and Tanaka, K. (2004) *Curr. Protein Pept. Sci.* 5, 177–184.
- (6) Ritchie, K.J. and Zhang, D.E. (2004) *Semin. Cell Dev. Biol.* 15, 237–246.
- (7) Kim, K.I. et al. (2002) *J. Cell Physiol.* 191, 257–268.
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- (9) Loeb, K.R. and Haas, A.L. (1992) *J. Biol. Chem.* 267, 7806–7813.
- (10) Zhao, C. et al. (2005) *Proc. Natl. Acad. Sci. USA* 102, 10200–10205.
- (11) Matunis, M.J. et al. (1996) *J. Cell Biol.* 135, 1457–1470.
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- (13) Gostissa, M. et al. (1999) *EMBO J.* 18, 6462–6471.
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- (15) Desterro, J.M. et al. (1998) *Mol. Cell* 2, 233–239.
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- (17) Xirodimas, D.P. et al. (2004) *Cell* 118, 83–97.
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- (20) Pagano, M. et al. (1995) *Science* 269, 682–685.
- (21) Yu, Z.K. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 11324–11329.
- (22) Pagano, M. (2004) *Mol. Cell* 14, 414–416.
- (23) Reed, S.I. (2003) *Nat. Rev. Mol. Cell Biol.* 4, 855–864.



Western blot analysis of recombinant GST-SUMO-1 protein (38 kDa), recombinant SUMO-2, recombinant SUMO-3, and extracts from C6 and PC12 cells, using **SUMO-2/3 (18H8) Rabbit mAb #4971** (upper) and **SUMO-1 Antibody #4930** (lower).



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

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- 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 per plate of 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 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.