

#4889 Store at -20°C

# Protein Folding and Stability Antibody Sampler Kit

1 Kit  
 (8 x 40 µl)



**Orders** ■ 877-616-CELL (2355)  
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**Support** ■ 877-678-TECH (8324)  
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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Products Included	Product #	Quantity	Applications	Species Cross-Reactivity	Mol. Wt.	Source
Skp1 Antibody	2156	40 µl	W, IF-IC, F	H, M, R	19 kDa	Rabbit
Skp2 Antibody	4358	40 µl	W, IP, IF-IC	H, Mk	48 kDa	Rabbit
ISG15 Antibody	2743	40 µl	W, E, F	H, M, Mk	15 kDa	Rabbit
NEDD8 Antibody	2745	40 µl	W, IP, IHC-P, IF-IC, F	H, M, R, Mk, (B, X, Z)	9 kDa	Rabbit
Ubiquitin (P4D1) Mouse mAb	3936	40 µl	W, IHC-P	All		Mouse
UBC3 Antibody	4997	40 µl	W, F	H, M, R	32 kDa	Rabbit
SUMO-1 Antibody	4930	40 µl	W, IP, IHC-P, IF-IC	H, M, R, Mk		Rabbit
SUMO-2/3 (18H8) Rabbit mAb	4971	40 µl	W, IF-IC	H, R, (M)		Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl				Goat
Anti-mouse IgG, HRP-linked Antibody	7076	50 µl				Goat

**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 mini-blot 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 com-

ponents, 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 Antibody detects endogenous levels of both free and conjugated ISG15 protein, but does not cross-react with other ubiquitin family members. NEDD8 Antibody detects endogenous levels of both free and conjugated NEDD8 protein, but does not cross-react with other ubiquitin family members. 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.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with synthetic peptides (KLH-coupled) corresponding to human SUMO-1 (#4930), UBC3 (#4997), Skp2 (#4358), Skp1 (#2156), NEDD8 (#2745), and ISG15 (#2743). 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 (KLH-coupled) 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.

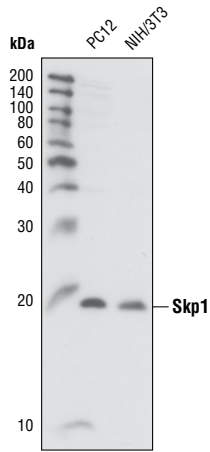
**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. *Do not aliquot the antibodies.*

**Recommended Antibody Dilutions:**  
 Western blotting 1:1000  
 See www.cellsignal.com for individual component dilutions and additional application protocols.

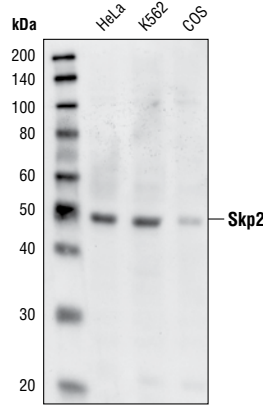
**Companion Products:**  
 CUL1 Antibody #4995  
 NEDD8 (19E3) Rabbit mAb #2754  
 UBC3B Antibody #4996  
 VHL Antibody #2738  
 Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071  
 Prestained Protein Marker, Broad Range (Premixed Format) #7720  
 Biotinylated Protein Ladder #7721  
 Skp1 Antibody #2156

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIATM  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected  
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

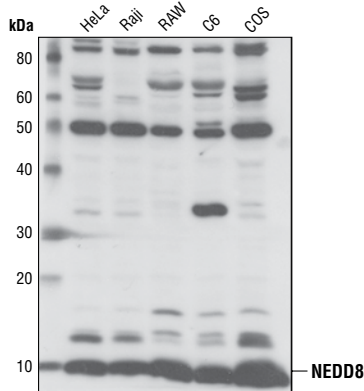
© 2008 Cell Signaling Technology, Inc. Rabbit Monoclonals produced using technology from Epitomics, Inc. under U.S. Patent No. 5,675,063.



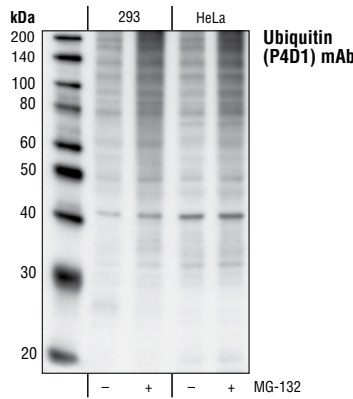
Western blot analysis of extracts from PC12 and NIH/3T3 cells using **Skp1 Antibody #2156**.



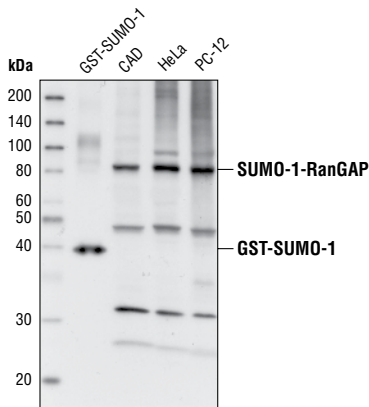
Western blot analysis of extracts from HeLa, K562 and COS cells using **Skp2 Antibody #4358**.



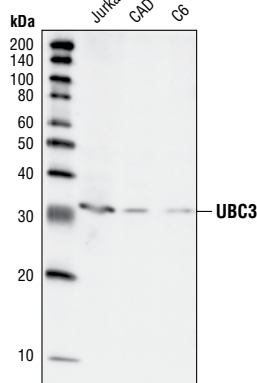
Western blot analysis of extracts from various cell types using **NEDD8 Antibody #2745**.



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



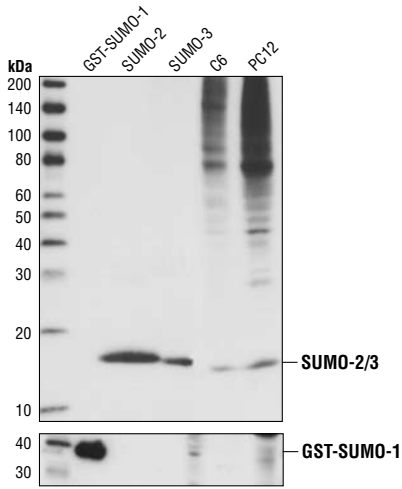
Western blot analysis of recombinant GST-SUMO-1 protein (38 kDa) and extracts from CAD, HeLa and PC-12 cells using **SUMO-1 Antibody #4930**.



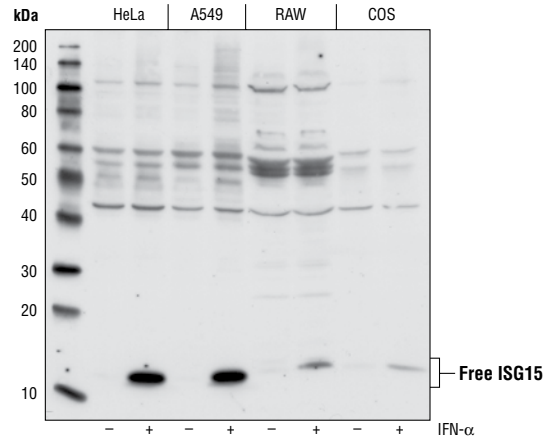
Western blot analysis of extracts from Jurkat, CAD and C6 cells using **UBC3 Antibody #4997**.

**Background References:**

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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 lysates from various cell types, untreated or IFN- $\alpha$  treated (1000 U/mL for 24 hours), using **ISG15 Antibody #2743**.

## 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<sup>®</sup>-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<sup>®</sup> 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 cm<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.