

Skp1 Antibody

✓ 100 µl
(10 western blots)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 09/01/10

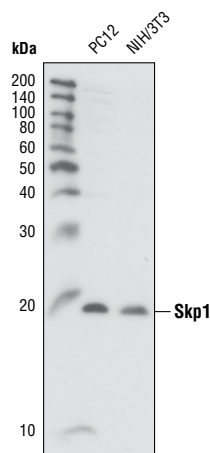
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

| Applications | Species Cross-Reactivity* | Molecular Wt. | Source |
|---------------------------|---------------------------|---------------|----------|
| W, IF-IC, F Endogenous | H, M, R | 19 kDa | Rabbit** |

Background: Ubiquitin can be covalently linked to many cellular proteins by the ubiquitination process, which targets 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 activation component E1; the activated ubiquitin is subsequently transferred to the ubiquitin-carrier protein E2 and then from E2 to ubiquitin ligase E3 for final delivery to the ε-NH₂ of the target protein lysine residue (1–3). Combinatorial interactions of different E2 and E3 proteins result in substrate specificity (4). Recent data suggest that activated E2 associates transiently with E3, and that the dissociation is a critical step for ubiquitination (5). Skp1 (S phase kinase-associated protein 1) is a critical scaffold protein of the SCF (Skp1/CUL1/F-box) E3 ubiquitin ligase protein complex. Various F-box proteins (e.g. β-TrCP, Skp2) mediate an interaction to both Skp1, via their defining and conserved domain of 40 amino acids, and to substrates to be ubiquitinated (e.g. β-catenin, p27) (4).

Specificity/Sensitivity: Skp1 Antibody detects endogenous levels of total Skp1 protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues of human Skp1. Antibodies are purified by protein A and peptide affinity chromatography.



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

Entrez-Gene ID #6500
Swiss-Prot Acc. #P63208

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 antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

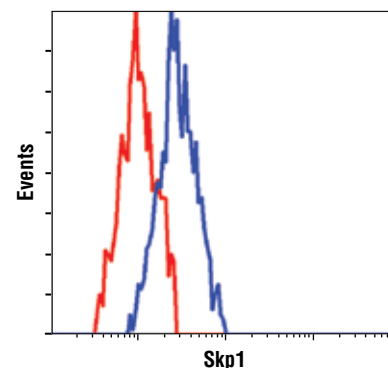
| | |
|----------------------------|--------|
| Western blotting | 1:1000 |
| Immunofluorescence (IF-IC) | 1:100 |
| Flow Cytometry | 1:100 |

For application specific protocols please see the web page for this product at www.cellsignal.com.

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

Background References:

- (1) Ciechanover, A. (1998) *EMBO J.* 17, 7151–60.
- (2) Hochstrasser, M. (2000) *Nat. Cell Biol.* 2, E153–7.
- (3) Hochstrasser, M. (2000) *Science* 289, 563–4.
- (4) DeSalle, L.M. and Pagano, M. (2001) *FEBS Lett.* 490, 179–89.
- (5) Deffenbaugh, A.E. et al. (2003) *Cell* 114, 611–22.

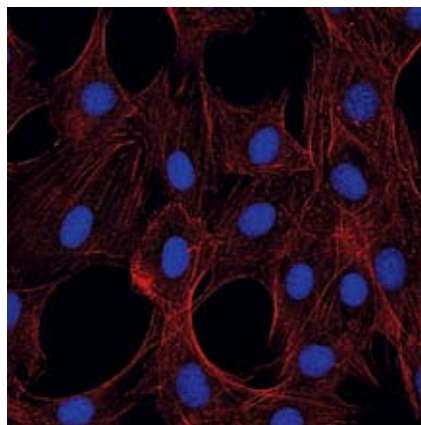
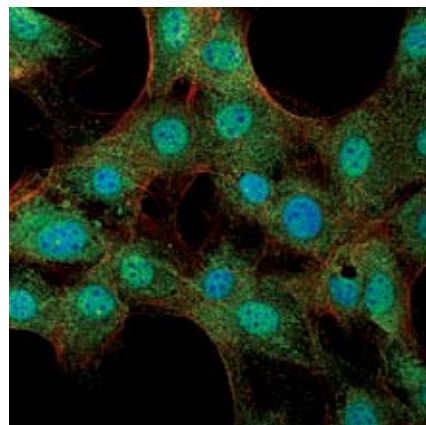


Flow cytometric analysis of NIH/3T3 cells, using Skp1 Antibody (blue) compared to a nonspecific negative control antibody (red).

Alexa Fluor® is a registered trademark of Molecular Probes, Inc.

DRAQ5® is a registered trademark of Biostatus Limited.

IMPORTANT: 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.



Confocal immunofluorescent images of NIH/3T3 cells labeled with Skp1 Antibody (green, left) compared to an isotype control (right). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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 Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.