Autophagy Antibody Sampler Kit

#4445

Store at -20°C

☑ 1 Kit
(7 x 40 µl)

Cell Signaling TECHNOLOGY®

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

rev. 05/16/11

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Beclin-1 (D40C5) Rabbit mAb	3495	40 µl	60 kDa	Rabbit IgG
LC3A (D50G8) XP™ Rabbit mAb	4599	40 µl	14, 16 kDa	Rabbit IgG
LC3B (D11) XP™ Rabbit mAb	3868	40 µl	14, 16 kDa	Rabbit IgG
Atg12 (D88H11) Rabbit mAb	4180	40 µl	16, 53 kDa	Rabbit IgG
Atg5 (D1G9) Rabbit mAb	8540	40 µl	55 kDa	Rabbit IgG
Atg7 Antibody	2631	40 µl	78 kDa	Rabbit IgG
Atg3 Antibody	3415	40 µl	40 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.

Description: The Autophagy Antibody Sampler Kit provides an economical means to investigate the molecular machinery of autophagy within the cell. The kit contains enough primary and secondary antibodies to perform four Western mini-blot experiments.

Background: Autophagy is a catabolic process for the autophagosomic-lysosomal degradation of bulk cytoplasmic contents (1,2). Autophagy is generally activated by conditions of nutrient deprivation but has also been associated with a number of physiological processes including development, differentiation, neurodegeneration, infection and cancer (3). The molecular machinery of autophagy was largely discovered in yeast and referred to as **a**u**t**opha**g**y-related (Atg) genes. Formation of the autophagosome involves an ubiquitin-like conjugation system in which Atg12 is covalently bound to Atg5 and targeted to autophagosome vesicles (4-6). This conjugation reaction is mediated by the ubiquitin-E1-like enzyme Atg7 and the E2-like enzyme Atg10 (7,8).

Specificity/Sensitivity: Each antibody in the Autophagy Antibody Sampler Kit detects its respective target at endogenous levels. Both the Atg5 and Atg12 rabbit monoclonal antibodies recognize the Atg12-Atg5 conjugate. The LC3A and LC3B rabbit monoclonal antibodies may cross-react with other LC3 isoforms. The LC3B rabbit monoclonal antibody has stronger reactivity with the type II form of LC3B by western immunoblot.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Thr72 of human Beclin-1, Pro187 of human Atg5, Ser36 of human Atg12, or near the amino termini of human LC3A and LC3B. Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues near the amino-termini of human Atg3 and Atg7. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Reggiori, F. and Klionsky, D.J. (2002) *Eukaryot Cell* 1, 11-21.

(2) Codogno, P. and Meijer, A.J. (2005) *Cell Death Differ* 12 Suppl 2, 1509-18.

(3) Levine, B. and Yuan, J. (2005) *J Clin Invest* 115, 2679-88.

(4) Mizushima, N. et al. (1998) *J Biol Chem* 273, 33889-92.

(5) Mizushima, N. et al. (1998) *Nature* 395, 395-8.

(6) Suzuki, K. et al. (2001) *EMBO J* 20, 5971-81.

(7) Tanida, I. et al. (1999) *Mol Biol Cell* 10, 1367-79.

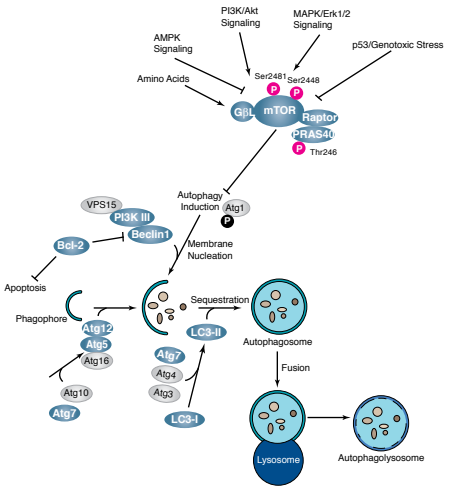
(8) Shintani, T. et al. (1999) *EMBO J* 18, 5234-41.

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:

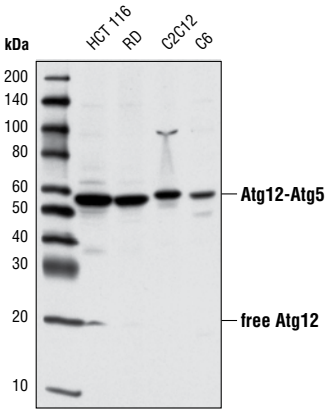
Western blotting	1:1000

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

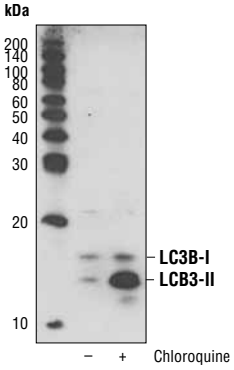


Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

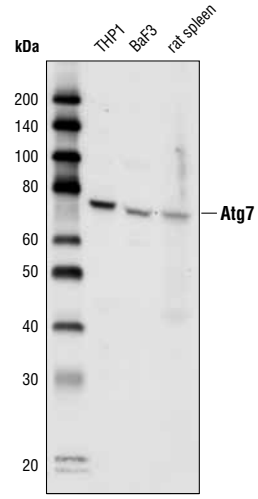
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.



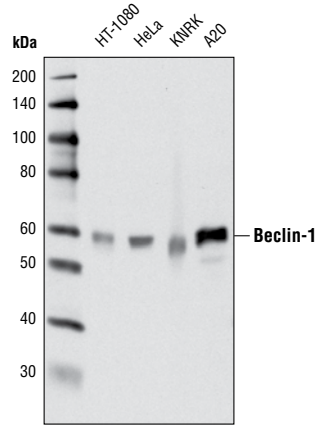
Western blot analysis of extracts from various cell lines using ***Atg12 (D88H11) Rabbit mAb #4180****.*



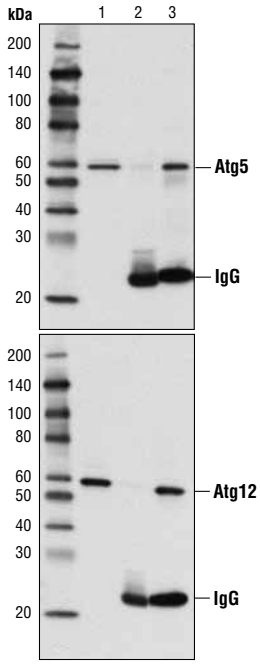
Western blot analysis of extracts from HeLa cells, untreated or chloroquine-treated (50 µM, overnight), using ***LC3B (D11) XP™ Rabbit mAb #3868****.*



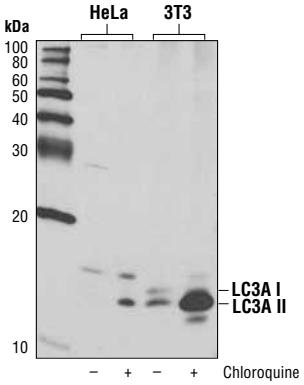
Western blot analysis of extracts from THP-1 and BaF3 cells and rat spleen using ***Atg7 Antibody #2631.***



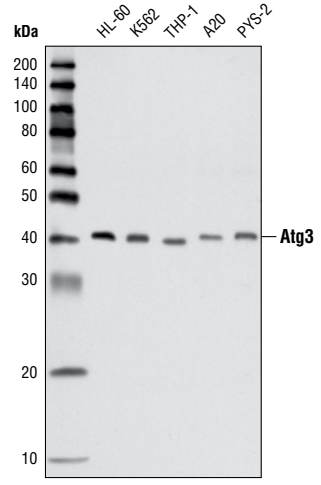
Western blot analysis of extracts from various cell lines using ***Beclin-1 (D40C5) Rabbit mAb #3495****.*



Immunoprecipitation of Atg5 from HT-1080 cells using ***Atg5 (D1G9) Rabbit mAb #8540****. Western blot detection was performed using the same antibody (upper), or with Atg12 (D88H11) Rabbit mAb #4180 (lower). Lane 1 is 10% input, lane 2 is precipitated with non-specific rabbit IgG, lane 3 is precipitated with* ***Atg5 (D1G9) Rabbit mAb #8540****.*



Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated or chloroquine-treated (50 µM, overnight), using ***LC3A (D50G8) XP™ Rabbit mAb #4599****.*



Western blot analysis of extracts from various cell lines using ***Atg3 Antibody #3415.***

Western Immunoblotting Protocol (Primary Antibody 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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

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 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.