

Autophagosome Marker Antibody Sampler Kit

✓ 1 Kit
(3 x 20 µl)



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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Atg12 Antibody (Human Specific)	2010	20 µl	16, 55 kDa	Rabbit IgG
LC3A (D50G8) XP® Rabbit mAb	4599	20 µl	14, 16 kDa	Rabbit IgG
LC3B (D11) XP® Rabbit mAb	3868	20 µl	14, 16 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 Autophagosome Marker Antibody Sampler Kit provides an economical means to investigate the accumulation of autophagosomes within the cell. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Autophagy is a catabolic process for the autophagosomal-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 autophagy-related (Atg) genes. Formation of the autophagosome involves a ubiquitin-like conjugation system in which Atg12 is covalently bound to Atg5 and targeted to autophagosomal vesicles (4-6). This conjugation reaction is mediated by the ubiquitin E1-like enzyme Atg7 and the E2-like enzyme Atg10 (7,8). Autophagy marker Light Chain 3 (LC3) was originally identified as a subunit of microtubule-associated proteins 1A and 1B (termed MAP1LC3) (9) and subsequently found to contain similarity to the yeast protein Apg8/Aut7/Cvt5 that is critical for autophagy (10). Three human LC3 isoforms (LC3A, LC3B, and LC3C) undergo post-translational modifications during autophagy (11-14). Cleavage of LC3 at the carboxy terminus immediately following synthesis yields the cytosolic LC3-I form. During autophagy, LC3-I is converted to LC3-II through lipidation by a ubiquitin-like system involving Atg7 and Atg3 that allows for LC3 to become associated with autophagic vesicles (11-15). The presence of LC3 in autophagosomes and the conversion of LC3 to the lower migrating form LC3-II have been used as indicators of autophagy (16).

Specificity/Sensitivity: Each antibody in the Autophagosome Marker Antibody Sampler Kit detects endogenous levels of its respective target. The Atg12 Antibody (Human Specific) recognizes free Atg12 and the Atg12-Atg5 conjugate. The LC3A and LC3B rabbit monoclonal antibodies may cross-react with other LC3 isoforms and have stronger reactivity with the type II form.

Source/Purification: Antibodies are produced by immunizing animals with synthetic peptides corresponding to residues near the amino termini of human Atg12, LC3A, or LC3B. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Storage: Supplied in 136 mM NaCl, 2.6 mM KCl, 12 mM sodium phosphate (pH 7.4) dibasic, 2 mg/ml BSA, and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

Recommended Antibody Dilutions:
Western blotting 1:1000

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

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.
- (9) Mann, S.S. and Hammarback, J.A. (1994) *J Biol Chem* 269, 11492-7.
- (10) Lang, T. et al. (1998) *EMBO J* 17, 3597-607.
- (11) Kabeya, Y. et al. (2000) *EMBO J* 19, 5720-8.
- (12) Wu, J. et al. (2006) *Biochem Biophys Res Commun* 339, 437-42.
- (13) He, H. et al. (2003) *J Biol Chem* 278, 29278-87.
- (14) Tanida, I. et al. (2004) *J Biol Chem* 279, 47704-10.
- (15) Ichimura, Y. et al. (2000) *Nature* 408, 488-92.
- (16) Kabeya, Y. et al. (2004) *J Cell Sci* 117, 2805-12.

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% v20 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.