Store at -20°C

#46486

Autophagy Induction (ULK1 Complex) Antibody Sampler Kit

1 Kit (6 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Atg101 (E1Z4W) Rabbit mAb	13492	20 µl	25 kDa	Rabbit IgG
Atg13 (D4P1K) Rabbit mAb	13273	20 µl	72 kDa	Rabbit IgG
FIP200 (D10D11) Rabbit mAb	12436	20 µl	200 kDa	Rabbit IgG
Phospho-ULK1 (Ser555) (D1H4) Rabbit mAb	5869	20 µl	140–150 kDa	Rabbit IgG
Phospho-ULK1 (Ser757) (D7O6U) Rabbit mAb	14202	20 µl	140–150 kDa	Rabbit IgG
ULK1 (D8H5) Rabbit mAb	8054	20 µl	150 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.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:

Western blotting	1:1000

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Description: The Autophagy Induction (ULK1 Complex) Antibody Sampler Kit provides an economical means of detecting target proteins in the ULK1 complex. The kit contains enough antibody to perform two western blot experiments per primary antibody.

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 autophagy-related (Atg) genes. ULK1, Atg13, and FIP200 form a complex that localizes to autophagic isolation membranes and regulates autophagosome biogenesis (4-6). mTOR phosphorylates both Atg13 and ULK1, suppressing ULK1 kinase activity and autophagy (5-7). Interaction between Atg101 and Atg13 can be important for the stability and basal phosphorylation of Atg13 and ULK1 (8,9). AMPK, activated during low nutrient conditions, directly phosphorylates ULK1 at multiple sites including Ser317, Ser555, and Ser777 (7,10). Conversely, mTOR, which is a regulator of cell growth and is an inhibitor of autophagy, phosphorylates ULK1 at Ser757 and disrupts the interaction between ULK1 and AMPK (7).

Specificity/Sensitivity: ULK1 (D8H5) Rabbit mAb, Atg13 (D4P1K) Rabbit mAb, FIP200 (D10D11) Rabbit mAb, and Atg101 (E1Z4W) Rabbit mAb recognize total endogenous levels of the corresponding target proteins irrespective of phosphorylation state. Phospho-ULK1 (Ser555) (D1H4) Rabbit mAb detects endogenous levels of ULK1 only when phosphorylated at Ser555 of mouse ULK1 (equivalent to Ser556 of human ULK1). Bands of unknown origin are detected between 90 and 100 kDa. Phospho-ULK1 (Ser757) (D7O6U) Rabbit mAb recognizes endogenous levels of ULK1 protein only when phosphorylated at Ser757 of mouse ULK1 (equivalent to Ser758 of human ULK1).

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Arg600 of human ULK1 protein, residues surrounding Asp462 of human Atg13 protein, residues surrounding Val177 of human Atg101 protein, or residues near the carboxy terminus of human FIP200 protein. Phospho-specific monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser555 of mouse ULK1 protein (equivalent to Ser556 of human ULK1) or residues surrounding Ser757 of mouse ULK1 protein (equivalent to Ser758 of human ULK1).

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) Ganley, I.G. et al. (2009) *J Biol Chem* 284, 12297-305.

(5) Hosokawa, N. et al. (2009) *Mol Biol Cell* 20, 1981-91.

(6) Jung, C.H. et al. (2009) *Mol Biol Cell* 20, 1992-2003.

(7) Kim, J. et al. (2011) *Nat Cell Biol* 13, 132-41.

(8) Mercer, C.A. et al. (2009) *Autophagy* 5, 649-62.

(9) Hosokawa, N. et al. (2009) *Autophagy* 5, 973-9.

(10) Egan, D.F. et al. (2011) *Science* 331, 456-61.

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

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.