

Store at  
-20°C

#85493

# ULK1 Substrate Antibody Sampler Kit

1 Kit  
(9 x 20 µl)

**Support:** +1-978-867-2388 (U.S.)  
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New 10/18

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
P-Atg14 (S29) (D4B8M) Rabbit mAb	92340	20 µl	65 kDa	Rabbit IgG
Atg14 (D1A1N) Rabbit mAb	96752	20 µl	65 kDa	Rabbit IgG
P-Beclin-1 (S15) (D4B7R) Rabbit mAb	84966	20 µl	60 kDa	Rabbit IgG
Beclin-1 (D40C5) Rabbit mAb	3495	20 µl	60 kDa	Rabbit IgG
P-Atg13 (S355) (D6J1W) Rabbit mAb	26839	20 µl	72 kDa	Rabbit IgG
Atg13 (D4P1K) Rabbit mAb	13273	20 µl	72 kDa	Rabbit IgG
ULK1 (D8H5) Rabbit mAb	8054	20 µl	150 kDa	Rabbit IgG
P-ULK1 (S757) (D7O6U) Rabbit mAb	14202	20 µl	140-150 kDa	Rabbit IgG
P-ULK1 (S555) (D1H4) Rabbit mAb	5869	20 µl	140-150 kDa	Rabbit IgG
Anti-Rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The ULK1 Substrate Antibody Sampler Kit provides an economical means of detecting the activity of ULK1 using phospho-specific and control antibodies. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

**Background:** Two related serine/threonine kinases, UNC-51-like kinase 1 and 2 (ULK1, ULK2), were discovered as mammalian homologs of the *C. elegans* gene UNC-51 in which mutants exhibited abnormal axonal extension and growth (1-4). Both proteins are widely expressed and contain an amino-terminal kinase domain followed by a central proline/serine rich domain and a highly conserved carboxy-terminal domain. The roles of ULK1 and ULK2 in axon growth have been linked to studies showing that the kinases are localized to neuronal growth cones and are involved in endocytosis of critical growth factors, such as NGF (5). Yeast two-hybrid studies found ULK1/2 associated with modulators of the endocytic pathway, SynGAP and syntenin (6). Structural similarity of ULK1/2 has also been recognized with the yeast autophagy protein Atg1/Atg13 (7). Knockdown experiments using siRNA demonstrated that ULK1 is essential for autophagy (8), a catabolic process for the degradation of bulk cytoplasmic contents (9,10). It appears that Atg1/ULK1 can act as a convergence point for multiple signals that control autophagy (11), and can bind to several autophagy-related (Atg) proteins, regulating phosphorylation states and protein trafficking (12-16). AMPK, activated during low nutrient conditions, directly phosphorylates ULK1 at multiple sites, including Ser317, Ser555, and Ser777 (17,18). 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 (17). ULK1 has been shown to phosphorylate several targets in the autophagy pathway, including Ser29 of Atg14, Ser15 of Beclin-1, and Ser355 of Atg13 (19-22).

**Specificity/Sensitivity:** Phospho-Beclin-1 (Ser15) (D4B7R) Rabbit mAb and Phospho-Atg13 (Ser555) (E6H1W) Rabbit mAb are only recommended for transfected levels of their specific targets. Phospho-Atg13 (Ser555) (E6H1W) Rabbit mAb can weakly detect endogenous levels of phosphorylated Atg13, but also reacts with a band of unknown origin at 25 kDa. All other antibodies in the kit can detect endogenous levels of their specific targets. Phospho-ULK1 (Ser555) (D1H4) Rabbit mAb detects a band of unknown origin between 90 and 100 kDa.

**Source/Purification:** Monoclonal antibodies are produced by immunizing rabbits with synthetic peptides corresponding to residues surrounding Arg70 of human Atg14, Thr72 of human Beclin-1, Asp462 of human Atg13, and Arg600 of human ULK1. Phosphorylation-specific monoclonal antibodies are produced by immunizing rabbits with synthetic phospho-peptides corresponding to Ser29 of Atg14, Ser15 of human Beclin-1, Ser355 of human Atg13 (Ser318 of isoform 2 of Atg13), Ser555 of mouse ULK1 (equivalent to Ser556 of human ULK1), and Ser757 of mouse ULK1 (equivalent to Ser758 of human ULK1).

**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 antibody.

#### Background References:

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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.**

## 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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH<sub>2</sub>O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH<sub>2</sub>O, mix.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

### B. Protein Blotting

**A general protocol for sample preparation.**

- 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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- 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.
- Electrotransfer to nitrocellulose membrane (#12369).

### 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.

#### I. Membrane Blocking

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

#### II. Primary Antibody Incubation

- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

### D. Detection of Proteins

- 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.
- 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.

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