Store at 4°C

PathScan® Phospho-ULK1 (Ser757) Sandwich ELISA Kit

#93778

1 Kit
(96 assays)

Support: +1-978-867-2388 (U.S.)
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Entrez-Gene ID #8408
UniProt ID #075385

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

Species Cross-Reactivity: H, M

Description: The PathScan® Phospho-ULK1 (Ser757) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of ULK1 protein phosphorylated at Ser757. Incubation of cell lysates and detection antibody on the coated microwell plate forms a sandwich with phospho-ULK1 (Ser757) in a single step. The plate is then extensively washed and TMB reagent is added for signal development. The magnitude of absorbance for the developed color is proportional to the quantity of phospho-ULK1 (Ser757).

*Antibodies in this kit are custom formulations specific to kit.

Specificity/Sensitivity: The PathScan® Phospho-ULK1 (Ser757) Sandwich ELISA Kit detects endogenous levels of ULK1 protein phosphorylated at Ser757. The kit sensitivity is shown in Figure 1. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

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/Apg1 (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 an inhibitor of autophagy, phosphorylates ULK1 at Ser757 and disrupts the interaction between ULK1 and AMPK (17).

Product Includes	Item #	Kit Quantity	Color	Storage Temp
Phospho-ULK1 (Ser757) Rabbit mAb Coated Microwells*	68745	96 tests		4°C
ULK1 Rabbit Detection mAb	53531	1 each	Red (Lyophilized)	4°C
HRP Diluent	13515	5.5 mL	Red	4°C
TMB Substrate	7004	11 mL		4°C
STOP Solution	7002	11 mL		4°C
Sealing Tape	54503	2 each		4°C
ELISA Wash Buffer (20X)	9801	25 mL		4°C
Cell Lysis Buffer (10X)	9803	15 mL		-20°C

*12 8-well modules – Each module is designed to break apart for 8 tests.
**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

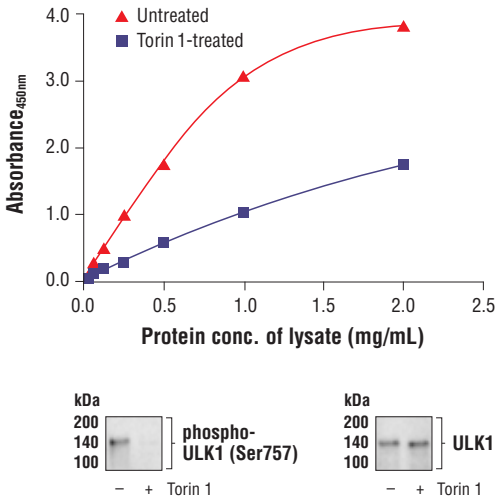

Figure 1. Treatment of A-172 cells with the mTOR inhibitor Torin 1 inhibits phosphorylation of ULK1 at Ser757. The relationship between lysate protein concentration from untreated and Torin 1-treated A-172 cells and the absorbance at 450 nm using the PathScan® Phospho-ULK1 (Ser757) Sandwich ELISA Kit #93778 is shown in the upper figure. The corresponding western blots using phospho-ULK1 (Ser757) antibody (left panel) and ULK1 antibody (right panel) are shown in the lower figure. A-172 cells were treated with Torin 1 (250 nM) for 5 hours at 37°C and then lysed.

Background References:

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(16) Hara, T. et al. (2008) *J Cell Biol* 181, 497-510.
(17) Kim, J. et al. (2011) *Nat Cell Biol* 13, 132-41.
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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.

#93778

PathScan® Sandwich ELISA Protocol

(One-Step Test Procedure)

NOTE: This protocol is for PathScan® kits that use an HRP directly conjugated to the detection antibody (1-step method), rather than a 2-step method where the detection antibody and a secondary-HRP are added sequentially.

Refer to product-specific datasheets for assay incubation temperature.

A Solutions and Reagents

NOTE: Prepare solutions with deionized/purified water or equivalent.

1. **Microwell strips:** Bring all to room temperature before opening bag/use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack and stored at 4°C.
2. **Detection Antibody:** Reconstitute lyophilized Detection Antibody (red colored cake) with 5.5 mL HRP Diluent. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. For best results, use immediately following antibody reconstitution. Unused reconstituted Detection Antibody may be stored for up to 4 weeks at 4°C, although there may be some loss of signal compared to freshly reconstituted antibody.
3. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the Detection Antibody that is linked to HRP.
4. **1X ELISA Wash Buffer:** Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) to 1X with deionized water.
5. **1X Cell Lysis Buffer:** Prepare by diluting 10X Cell Lysis Buffer #9803 to 1X with deionized water. This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: When using to prepare cell lysates, add Protease/Phosphatase Inhibitor Cocktail (#5872, not supplied) and 1 mM phenylmethyl-sulfonyl fluoride (PMSF, #8553, not supplied) immediately before use.
6. **TMB Substrate (#7004):** Bring to room temperature before use.
7. **STOP Solution (#7002):** Bring to room temperature before use.

B Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 mL ice-cold 1X Cell Lysis Buffer including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/mL. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5-10 mL ice-cold 1X PBS.
3. Cells harvested from 50 mL of growth media can be lysed in 2.0 mL of 1X Cell Lysis Buffer including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

C Test Procedure

NOTE: Equilibrate all materials and prepared reagents to room temperature prior to running the assay.

1. Prepare all reagents as indicated above (Section A).
2. Samples should be undiluted or diluted with 1X Cell Lysis Buffer to a 2X protein concentration in order to achieve a final 1X protein concentration upon addition of the Detection Antibody. Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical results across a range of lysate concentration points.
3. Add 50 µL of each sample to the appropriate wells.
4. Add 50 µL of the Detection Antibody to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm (moderate agitation).
6. Gently remove the tape and wash wells:
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 1X Wash Buffer, 200 µL each time for each well.
   c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   d. Clean the underside of all wells with a lint-free tissue.
7. Add 100 µL of TMB Substrate to each well. Seal with tape and incubate the plate in the dark for 15 min at room temperature on a plate shaker (400 rpm, moderate agitation) or alternatively for 10 min at 37°C without shaking.
8. Add 100 µL of STOP Solution to each well. Shake gently for a few seconds.

   NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.
9. Read results:
   a. **Visual Determination:** Read within 30 min after adding STOP Solution.
   b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.