

Store at
4°C

PathScan® Phospho-Atg14 (Ser29) Sandwich ELISA Kit



#71484

1 Kit
(96 assays)Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/supportOrders: 877-616-2355 (U.S.)
orders@cellsignal.comEntrez-Gene ID #22863
UniProt ID #Q6ZNE5

New 06/21

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

Species Cross-Reactivity: H

Description: The PathScan® Phospho-Atg14 (Ser29) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Atg14 protein phosphorylated at Ser29. Incubation of cell lysates and detection antibody on the coated microwell plate forms a sandwich with Atg14 protein phosphorylated at Ser29 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 Atg14 protein phosphorylated at Ser29.

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

Specificity/Sensitivity: The PathScan® Phospho-Atg14 (Ser29) Sandwich ELISA Kit detects endogenous levels of Atg14 protein phosphorylated at Ser29. 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: 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 is also 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 is directed by a number of autophagy-related (Atg) genes. These proteins are involved in the formation of autophagosomes, cytoplasmic vacuoles that are delivered to lysosomes for degradation. The class III type phosphoinositide 3-kinase (PI3K) Vps34 regulates vacuolar trafficking and autophagy (4,5). Multiple proteins associate with Vps34, including p105/Vps15, Beclin-1, UVRAG, Atg14, and Rubicon, to determine Vps34 function (6-12). Atg14 and Rubicon were identified based on their ability to bind to Beclin-1 and participate in unique complexes with opposing functions (9-12). Rubicon, which localizes to the endosome and lysosome, inhibits Vps34 lipid kinase activity; knockdown of Rubicon enhances autophagy and endocytic trafficking (11,12). In contrast, Atg14 localizes to autophagosomes, isolation membranes and ER, and can enhance Vps34 activity. Knockdown of Atg14 inhibits starvation-induced autophagy (11,12).

The serine/threonine kinase ULK1 phosphorylates Atg14 at Ser29 to promote autophagosome formation (13).

Product Includes	Item #	Kit Quantity	Color	Storage Temp
Atg14 Rabbit mAb Coated Microwells*	37097	96 tests		4°C
Phospho-Atg14 (Ser29) Rabbit Detection mAb	57676	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).

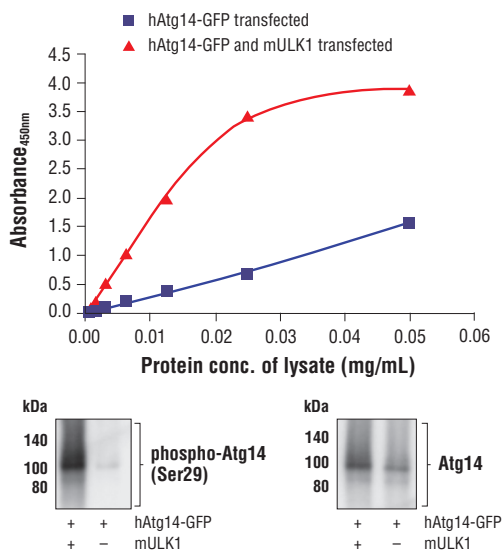


Figure 1. Atg14 is phosphorylated at Ser29 in 293T cells that were co-transfected with constructs expressing human Atg14 and mouse ULK1, but is not phosphorylated in cells transfected with only an Atg14 expression construct. The relationship between lysate protein concentration from Atg14/ULK1 co-transfected and Atg14-only transfected cells and the absorbance at 450 nm using the PathScan® Phospho-Atg14 (Ser29) Sandwich ELISA Kit #71484 is shown in the upper figure. The corresponding western blots using phospho-Atg14 (Ser29) antibody (left panel) and Atg14 antibody (right panel) are shown in the lower figure. 293T cells were either co-transfected with constructs expressing GFP-tagged human Atg14 protein (hAtg14-GFP) and mouse ULK1 (mULK1) or just transfected with a construct expressing GFP-tagged human Atg14 protein (hAtg14-GFP), and then lysed.

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) Corvera, S. (2001) *Traffic* 2, 859-66.
- (5) Yan, Y. and Backer, J.M. (2007) *Biochem Soc Trans* 35, 239-41.
- (6) Stack, J.H. et al. (1995) *J Cell Biol* 129, 321-34.
- (7) Zeng, X. et al. (2006) *J Cell Sci* 119, 259-70.
- (8) Liang, C. et al. (2006) *Nat Cell Biol* 8, 688-99.
- (9) Itakura, E. et al. (2008) *Mol Biol Cell* 19, 5360-72.
- (10) Sun, Q. et al. (2008) *Proc Natl Acad Sci U S A* 105, 19211-6.
- (11) Zhong, Y. et al. (2009) *Nat Cell Biol* 11, 468-76.
- (12) Matsunaga, K. et al. (2009) *Nat Cell Biol* 11, 385-96.
- (13) Park, J.M. et al. (2016) *Autophagy* 12, 547-64.

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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.

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.

- 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.
- 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.
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the Detection Antibody that is linked to HRP.
- 1X ELISA Wash Buffer:** Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) to 1X with deionized water.
- 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.
- TMB Substrate (#7004):** Bring to room temperature before use.
- STOP Solution (#7002):** Bring to room temperature before use.

B Preparing Cell Lysates

For adherent cells

- Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
- Remove media and rinse cells once with ice-cold 1X PBS.
- 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.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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

- Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10⁶ viable cells/mL. Treat cells by adding fresh media containing regulator for desired time.
- Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 mL ice-cold 1X PBS.
- 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.
- Sonicate lysates on ice.
- 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.

- Prepare all reagents as indicated above (Section A).
- 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.
- Add 50 µL of each sample to the appropriate wells.
- Add 50 µL of the Detection Antibody to each well.
- Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm (moderate agitation).
- Gently remove the tape and wash wells:
 - Discard plate contents into a receptacle.
 - Wash 4 times with 1X Wash Buffer, 200 µL each time for each well.
 - 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.
 - Clean the underside of all wells with a lint-free tissue.
- 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.
- 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.
- Read results:
 - Visual Determination:** Read within 30 min after adding STOP Solution.
 - Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.