Store at 4°C

#7955

PathScan® Phospho-AMPKα (Thr172) Sandwich ELISA Antibody Pair

Orders ■ 877-616-CELL (2355)
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Support ■ 877-678-TECH (8324)
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☑ 1 Kit
(4 X 96 assays)

rev. 07/10/18

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

Species Cross-Reactivity: H, M

Description: CST's PathScan® Phospho-AMPKα (Thr172) Sandwich ELISA Antibody Pair is offered as an economical alternative to our PathScan® Phospho-AMPKα-(Thr172) Sandwich ELISA Kit #7959. Capture and Detection antibodies (100X stocks) and Anti-Mouse IgG, HRP-linked Antibody (1000X stock) are supplied. Sufficient reagents are provided for 4 x 96 well ELISAs. The AMPKα Rabbit Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added followed by a Phospho-AMPKα (Thr172) Mouse Detection Antibody and Anti-Mouse IgG, HRP-linked Antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of Phospho-AMPKα (Thr172) protein.

Antibodies in kit are custom formulations specific to kit.

Reagents not supplied:

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween®20 (PBST-20X) #9809

Cell Lysis Buffer (1X) #7018

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer: 1X PBS/0.05% Tween®20, 1% BSA

96 Well Microplates**

Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592).

Notes: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

Background: AMP-activated protein kinase (AMPK) is highly conserved from yeast to plants and animals and plays a key role in the regulation of energy homeostasis (1). AMPK is a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits, each of which is encoded by two or three distinct genes (α1, 2; β1, 2; γ1, 2, 3) (2). The kinase is activated by an elevated AMP/ATP ratio due to cellular and environmental stress, such as heat shock, hypoxia and ischemia (1). The tumor suppressor LKB1, in association with accessory proteins STRAD and MO25, phosphorylates AMPKα at Thr172 in the activation loop and this phosphorylation is required for AMPK activation (3-5). AMPKα is also phosphorylated at Thr258 and Ser485 (for α1; Ser491 for α2). The upstream kinase and the biological significance of these phosphorylation events have yet to be elucidated (6). The β1 subunit is post-translationally modified by myristoylation and multi-site phosphorylation including Ser24/25, Ser96, Ser101, Ser108 and Ser182 (6,7). Phosphorylation at Ser108 of the β1 subunit seems to be required for the activation of AMPK enzyme, while phosphorylation at Ser24/25 and Ser182 affects AMPK localization (7). Several mutations in AMPKγ subunits have been identified, most of which are located in the putative AMP/ATP binding sites (CBS or Bateman domains). Mutations at these sites lead to reduction of AMPK activity and cause glycogen accumulation in heart or skeletal muscle (1,2). Accumulating evidence indicates that AMPK not only regulates the metabolism of fatty acids and glycogen, but also modulates protein synthesis and cell growth through EF2 and TSC2/mTOR pathways, as well as blood flow via eNOS/nNOS (1).

Product Includes	Item #	Volume	Cap Color	Storage Temp
AMPKα Capture Rabbit mAb (100X)	61108	400 µL	Pink	4°C
Phospho-AMPKα (Thr172) Detection Mouse mAb (100X)	31458	400 µL	Blue	4°C
Anti-mouse IgG, HRP-linked Antibody (1000X)	16736	40 µL	Yellow	–20°C

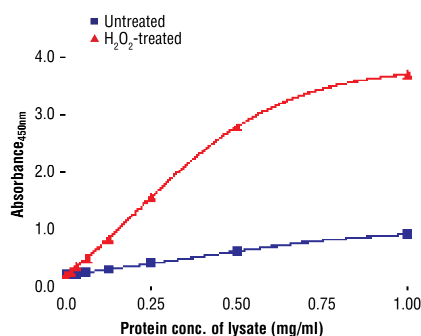

Figure 1. The relationship between the protein concentration of lysates from untreated and H_2O_2-treated C2C12 cells and the absorbance at 450 nm using the PathScan® Phospho-AMPKα (Thr172) Sandwich ELISA Antibody Pair #7955 is shown.

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

Background References:

(1) Hardie, D.G. (2004) *J Cell Sci* 117, 5479-87.

(2) Carling, D. (2004) *Trends Biochem Sci* 29, 18-24.

(3) Hawley, S.A. et al. (1996) *J Biol Chem* 271, 27879-87.

(4) Lizcano, J.M. et al. (2004) *EMBO J* 23, 833-43.

(5) Shaw, R.J. et al. (2004) *Proc Natl Acad Sci U S A* 101, 3329-35.

(6) Woods, A. et al. (2003) *J Biol Chem* 278, 28434-42.

(7) Warden, S.M. et al. (2001) *Biochem J* 354, 275-83.

U.S. Patent No. 5,675,063
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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

1. **Coating Buffer:** 1X PBS, (20X PBS #9808)
   3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4
2. **Wash Buffer:** 1X PBS/0.05% Tween®20, (20X PBST #9809)
3. **Blocking Buffer:** 1X PBS/0.05% Tween®20, 1% BSA
4. **1X Cell Lysis Buffer:**
   20 mM Tris-HCl (pH 7.5)
   150 mM NaCl
   1 mM disodium EDTA
   1 mM EGTA
   1% Triton
   20 mM sodium pyrophosphate
   25 mM sodium fluoride
   1 mM β-glycerophosphate
   1 mM Na_3VO_4
   1 µg/ml leupeptin
5. **TMB Substrate:** (TMB Substrate #7004)
6. **STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

1. Rinse microplate with dH_2O. Add 200 µl of dH_2O and discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 µl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
3. **After overnight coating, gently uncover plate and wash wells:**
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 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.
   c. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
5. After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5–1.0 ml ice-cold 1X PathScan® Sandwich ELISA Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 2 minutes.
4. Collect cell lysate into new tubes. This cell lysate solution can be used directly in this ELISA Kit, or stored at –80°C in single-use aliquots.

D Test Procedure

1. Lysates can be used undiluted or diluted in Blocking Buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
2. Wash plate as in Coating Procedure, Step 3.
3. Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 µl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hour.
4. Plate is washed as in Coating Procedure, Step 3.
5. Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30 minutes.
6. Wash plate as in Coating Procedure, Step 3.
7. Add 100 µl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
8. Add 100 µl of STOP Solution per well.
9. Read plate on a microplate reader at Absorbance 450 nm.

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