

Store at  
4°C

# PathScan® Total AMPK $\alpha$ Sandwich ELISA Kit

www.cellsignal.com

#7961C

1 Kit (96 assays)

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

## Species Cross-Reactivity: H, M, R, Hm, Mk

**Description:** PathScan® Total AMPK $\alpha$  Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of AMPK $\alpha$ . An AMPK $\alpha$  Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, AMPK $\alpha$  (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a biotinylated AMPK $\alpha$  Rabbit Detection mAb is added to detect the captured phospho and nonphospho AMPK $\alpha$  protein. A HRP-linked streptavidin antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of total AMPK $\alpha$ .

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

**Specificity/Sensitivity:** PathScan® Total AMPK $\alpha$  Sandwich ELISA Kit detects endogenous levels of AMPK $\alpha$  protein as shown in Figure 1. The kit sensitivity is shown in Figure 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

**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  $\alpha$  subunit and regulatory  $\beta$  and  $\gamma$  subunits, each of which is encoded by two or three distinct genes ( $\alpha 1, 2; \beta 1, 2; \gamma 1, 2, 3$ ) (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 AMPK $\alpha$  at Thr172 in the activation loop, and this phosphorylation is required for AMPK activation (3-5). AMPK $\alpha$  is also phosphorylated at Thr258 and Ser485 (for  $\alpha 1$ ; Ser491 for  $\alpha 2$ ). The upstream kinase and the biological significance of these phosphorylation events have yet to be elucidated (6). The  $\beta 1$  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  $\beta 1$  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 AMPK $\gamma$  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 #	Kit Quantity	Color	Storage Temp
AMPK $\alpha$ Rabbit mAb Coated Microwells*	60178	96 tests		4°C
AMPK $\alpha$ Rabbit Detection mAb (Biotinylated)	13954	1 each	Green (Lyophilized)	4°C
HRP-linked Streptavidin (ELISA Formulated)	11805	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
ELISA Sample Diluent	11083	25 ml	Blue	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.

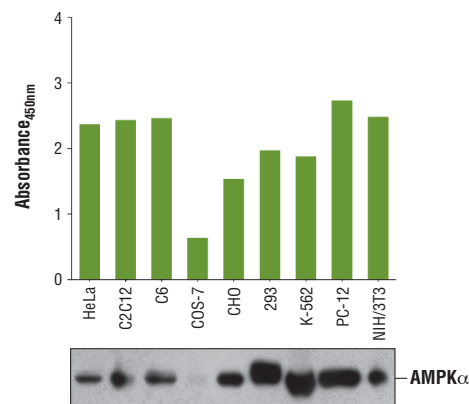


Figure 1. AMPK $\alpha$  protein is detected at varied levels from multiple cell lines using the PathScan® Total AMPK $\alpha$  Sandwich ELISA Kit #7961. The absorbance readings at 450 nm are shown in the top figure, while corresponding western blots using AMPK $\alpha$  (D63G4) Rabbit mAb #5832 are shown in the bottom figure.

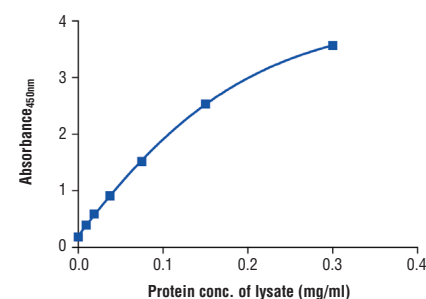


Figure 2. The relationship between protein concentration of lysates from HeLa cells and the absorbance at 450 nm as detected by the PathScan® Total AMPK $\alpha$  Sandwich ELISA Kit #7961 is shown. HeLa cells (85% confluence) were harvested and then lysed.

## 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 USA* 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.

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## PathScan® Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

### A Solutions and Reagents

**NOTE:** Prepare solutions with purified water.

- Microwell strips:** Bring all to room temperature before use.
- Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- HRP-Linked Antibody\*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803 or 1X Cell Lysis Buffer #7018: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) immediately before use.
- TMB Substrate** (#7004).
- STOP Solution** (#7002).

\*Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.

### 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 plus 1 mM PMSF 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 × 10<sup>6</sup> 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 plus 1 mM PMSF.
- 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

- After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
- Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). 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 kit assay results across a range of lysate concentration points.
- Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
- 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 reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
- Repeat wash procedure (Section C, Step 4).
- Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
- Repeat wash procedure (Section C, Step 4).
- Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
- 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.