



#7792 Store at +4C

PathScan® Phospho-AMPKα (Thr172) Chemiluminescent Sandwich ELISA Kit

Orders: 877-616-CELL (2355)
orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com
cellsignal.com

1 Kit (96 assays)

Species Cross Reactivity:
H M

UniProt ID:
#Q13131, #P54646

Entrez-Gene Id:
#5562, #5563

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Quantity	Color	Storage Temp
AMPKα Rabbit mAb Coated Microwells	36066	96 tests		+4C
Phospho-AMPKα (Thr172) Mouse Detection mAb	9881	1 ea	Green (Lyophilized)	+4C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 ea	Red (Lyophilized)	+4C
Detection Antibody Diluent	13339	5.5 ml	Green	+4C
HRP Diluent	13515	5.5 ml	Red	+4C
Luminol/Enhancer Solution	84850	3 ml		RT
Stable Peroxide Buffer	42552	3 ml		RT
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml		+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
PathScan® Sandwich ELISA Lysis Buffer (1X)	7018	30 ml		-20C

Kit contents scale proportionally with size, except sealing tape.

Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

The PathScan® Phospho-AMPKα (Thr172) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-AMPKα (Thr172) protein with a chemiluminescent readout. Chemiluminescent ELISAs often have a wider dynamic range and higher sensitivity than conventional chromogenic detection. This chemiluminescent ELISA, which is offered in low volume microplates, shows increased signal and sensitivity while using smaller samples. An AMPKα Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, AMPKα (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Phospho-AMPKα (Thr172) Mouse Detection Antibody is added to detect the captured phospho-AMPKα (Thr172) protein. HRP-linked, anti-mouse antibody is then used to recognize the bound detection antibody. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of phospho-AMPKα (Thr172) protein.

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

Specificity/Sensitivity

PathScan® Phospho-AMPKα (Thr172) Chemiluminescent Sandwich ELISA Kit detects endogenous levels of phospho-AMPKα (Thr172), as 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

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 AMPK activation, 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).

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.

Trademarks and Patents

Cell Signaling Technology is a trademark of Cell Signaling Technology, Inc.

PathScan is a registered trademark of Cell Signaling Technology, Inc.

All other trademarks are the property of their respective owners. Visit cellsignal.com/trademarks for more information.

Limited Uses

Except as otherwise expressly agreed in a writing signed by a legally authorized representative of CST, the following terms apply to Products provided by CST, its affiliates or its distributors. Any Customer's terms and conditions that are in addition to, or different from, those contained herein, unless separately accepted in writing by a legally authorized representative of CST, are rejected and are of no force or effect.

Products are labeled with For Research Use Only or a similar labeling statement and have not been approved, cleared, or licensed by the FDA or other regulatory foreign or domestic entity, for any purpose. Customer shall not use any Product for any diagnostic or therapeutic purpose, or otherwise in any manner that conflicts with its labeling statement. Products sold or licensed by CST are provided for Customer as the end-user and solely for research and development uses. Any use of Product for diagnostic, prophylactic or therapeutic purposes, or any purchase of Product for resale (alone or as a component) or other commercial purpose, requires a separate license from CST. Customer shall (a) not sell, license, loan, donate or otherwise transfer or make available any Product to any third party, whether alone or in combination with other materials, or use the Products to manufacture any commercial products, (b) not copy, modify, reverse engineer, decompile, disassemble or otherwise attempt to discover the underlying structure or technology of the Products, or use the Products for the purpose of developing any products or services that would compete with CST products or services, (c) not alter or remove from the Products any trademarks, trade names, logos, patent or copyright notices or markings, (d) use the Products solely in accordance with CST Product Terms of Sale and any applicable documentation, and (e) comply with any license, terms of service or similar agreement with respect to any third party products or services used by Customer in connection with the Products.

#7792

PathScan® Phospho-AMPKα (Thr172) Chemiluminescent Sandwich ELISA Kit

ELISA Chemiluminescent (Lyophilized)

NOTE: Refer to product-specific datasheets for assay incubation temperature. This chemiluminescent ELISA is offered in low volume microplates. Only 50 µl of samples or reagents are required in each microwell.

A. Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 0.5 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 0.5 ml volume of reconstituted Detection Antibody to 5.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
3. **HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 0.5 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 0.5 ml volume of reconstituted HRP-Linked Antibody to 5.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody.
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody.
6. **Sample Diluent:** Blue colored diluent for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
8. **Cell Lysis Buffer:** PathScan® Sandwich ELISA Lysis Buffer (1X) #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.
9. **Luminol/Enhancer Solution and Stable Peroxide Buffer.**

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

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 to 1 ml ice-cold PathScan® Sandwich ELISA Lysis Buffer (1X) #7018 plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 2 min.
4. Collect cell lysate in a clean tube.
5. Centrifuge for 10 min (14,000 x g) at 4°C and transfer the supernatant to a new tube. Store supernatant 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 10⁶ 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 plus 1 mM PMSF.
4. Resuspend the cell pellet and incubate the tube for 2 min.
5. Microcentrifuge for 10 min (x14,000 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

1. 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.
2. 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.

3. Add 50 μ l 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 room temperature. Alternatively, the plate can be incubated overnight at 4°C.
4. Gently remove the tape and wash wells:
 1. Discard plate contents into a receptacle.
 2. Wash 4 times with 1X Wash Buffer, 150 μ l each time for each well.
 3. 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.
 4. Clean the underside of all wells with a lint-free tissue.
5. Add 50 μ l of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at room temperature for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 50 μ l of reconstituted HRP-linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate at room temperature for 30 min.
8. Repeat wash procedure (Section C, Step 4).
9. Prepare Detection Reagent Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50 μ l of the Detection Reagent Working Solution to each well.
11. Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nm within 1–10 min following addition of the substrate. Optimal signal intensity is achieved when read within 10 min.

posted November 2013

revised January 2016