#12116C Store at 4°C

PathScan® Phospho-Acetyl-CoA Carboxylase (Ser79) Chemiluminescent Sandwich ELISA Kit

☑ 1 Kit
(96 assays)
Low volume microplate

Cell Signaling TECHNOLOGY®

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rev. 08/25/14

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

Entrez-Gene ID #31, 32
Swiss-Prot Acc. #Q13085, O00763

Species Cross-Reactivity: H

Description: The PathScan® Phospho-Acetyl-CoA Carboxylase (Ser79) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of acetyl-CoA carboxylase (ACC) protein phosphorylated at Ser79 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. A Phospho-ACC (Ser79) Rabbit Antibody has been coated onto the microwells. After incubation with cell lysates, phospho-ACC protein is captured by the coated antibody. Following extensive washing, an ACC Mouse Detection mAb is added to detect the captured ACC protein. Anti-mouse IgG, HRP-linked 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-ACC (Ser79) protein.

Antibodies in kit are custom formulations specific to kit.

Background: Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA (1). It is the key enzyme in the biosynthesis and oxidation of fatty acids (1). In rodents, the 265 kDa ACC1 (ACCα) form is primarily expressed in lipogenic tissues, while 280 kDa ACC2 (ACCβ) is the main isoform in oxidative tissues (1,2). However, in humans, ACC2 is the predominant isoform in both lipogenic and oxidative tissues (1,2). Phosphorylation by AMPK at Ser79 or by PKA at Ser1200 inhibits the enzymatic activity of ACC (3). ACC is a potential target of anti-obesity drugs (4,5).

Specificity/Sensitivity: PathScan® Phospho-Acetyl-CoA Carboxylase (Ser79) Chemiluminescent Sandwich ELISA Kit recognizes endogenous levels of phospho-ACC (Ser79) in human cells. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background References:

(1) Castle, J.C. et al. (2009) *PLoS One* 4, e4369.

(2) Kreuz, S. et al. (2009) *Diabetes Metab Res Rev* 25, 577-86.

(3) Ha, J. et al. (1994) *J Biol Chem* 269, 22162-8.

(4) Abu-Elheiga, L. et al. (2001) *Science* 291, 2613-6.

(5) Levert, K.L. et al. (2002) *J Biol Chem* 277, 16347-50.

Products Included	Volume	Color
Phospho-ACC (Ser79) Rabbit Antibody Coated Microwells*	96 tests	
ACC Mouse Detection mAb	1 each	Green (Lyophilized)
Anti-mouse IgG, HRP-Linked Antibody	1 each	Red (Lyophilized)
Detection Antibody Diluent	5.5 ml	Green
HRP Diluent	5.5 ml	Red
Luminol/Enhancer Solution	3 ml	Colorless
Stable Peroxide Buffer	3 ml	Colorless
Sealing Tape	2 sheets	
20X ELISA Wash Buffer	25 ml	Colorless
ELISA Sample Diluent	25 ml	Blue
10X Cell Lysis Buffer #9803**	15 ml	Yellowish

Low volume microplate * 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).

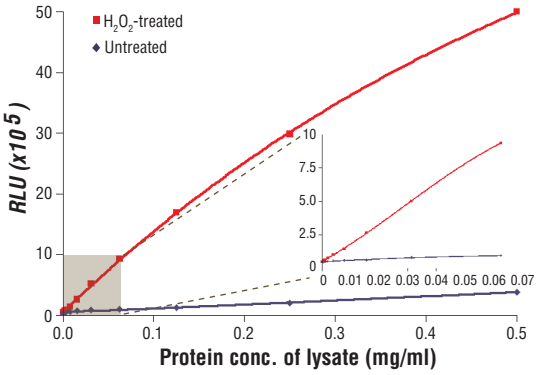

Figure 1. Relationship between protein concentration of lysates from untreated and H2O2-treated Hep G2 cells and immediate light generation with chemiluminescent substrate is shown. Hep G2 cells (80-90% confluent) were treated with H_2O_2 (10 mM, 10 min) and lysed with PathScan® Sandwich ELISA Lysis Buffer (1X) #7018. Graph inset corresponding to the shaded area shows high sensitivity and a linear response at the low protein concentration range.

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

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 full 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 full 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 (5.5 ml provided).
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (5.5 ml provided).
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:** 10X Cell Lysis Buffer #9803: 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 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.
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 plus 1 mM PMSF.
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

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 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 room temperature. Alternatively, the plate can be incubated overnight at 4°C.
4. Gently remove the tape and wash wells:
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 1X Wash Buffer, 150 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.
5. Add 50 µl 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 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 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 µl 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.*