Version 16-10/24

User's Manual

Phospho Acetyl-CoA Carboxylase Ser79 ELISA Kit

REF **DEIASL625**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Phospho-Acetyl-CoA Carboxylase (Ser79) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of acetyl-CoA carboxylase (ACC) when phosphorylated at Ser79.

General Description

Acetyl-CoA carboxylase (ACC) catalyzes the pivotal step of the fatty acid synthesis pathway. The 265 kDa ACCα (ACC1) is the predominant isoform found in liver, adipocytes and mammary gland, while the 280 kDa ACCβ (ACC2) is the major isoform in skeletal muscle and heart (1). Phosphorylation by AMPK at Ser79 or by PKA at Ser1200 inhibits the enzymatic activity of ACC (2). ACC is a potential target of anti-obesity drugs (3,4).

Principles of Testing

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. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for the developed color is proportional to the quantity of ACC phosphorylated at Ser79. Antibodies in kit are custom formulations specific to kit.

Reagents And Materials Provided

1. Phospho-ACC (Ser79) Rabbit mAb Coated Microwells*: 96 tests. 4°C
2. Acetyl-CoA Carboxylase Mouse Detection mAb: 1 each, Green (Lyophilized). 4°C
3. Anti-Mouse IgG, HRP-linked Antibody (ELISA Formulated): 1 each, Red (Lyophilized). 4°C
4. Detection Antibody Diluent: 11 ml, Green. 4°C
5. HRP Diluent: 11 ml, Red. 4°C
6. TMB Substrate: 11 ml. 4°C
7. STOP Solution: 11 ml. 4°C
8. Sealing Tape: 2 sheets. 4°C
9. ELISA Wash Buffer (20X): 25 ml. 4°C
10. ELISA Sample Diluent: 25 ml, Blue. 4°C
11. Sandwich ELISA Lysis Buffer (1X): 30 ml. -20°C

*12 8-well modules – Each module is designed to break apart for 8 tests.

Specimen Collection And Preparation

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 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. 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.

Reagent Preparation

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 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.

3. 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.

4. Detection Antibody Diluent: Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).

5. HRP Diluent: Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).

6. Sample Diluent: Blue colored diluent provided for dilution of cell lysates.

7. 1X Wash Buffer: Prepare by diluting 20X Wash Buffer (included in each Sandwich ELISA Kit) in purified water.

8. Cell Lysis Buffer: 1X Cell Lysis Buffer: 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. TMB Substrate.

10. STOP Solution.

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

Assay 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 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 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.
4. Gently remove the tape and wash wells:

a. Discard plate contents into a receptacle.

b. Wash 4 times with 1X Wash Buffer, 200 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 100 µl of reconstituted Detection Antibody (green color) to each well. Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure.
7. Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure.
9. 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.
10. 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.

11. Read results.

a. **Visual Determination**: Read within 30 min after adding STOP Solution.

b. **Spectrophotometric Determination**: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.

Sensitivity

Phospho-Acetyl-CoA Carboxylase (Ser79) Sandwich ELISA Kit detects endogenous levels of ACC protein when phosphorylated at Ser79 as shown in Figure 1. 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.

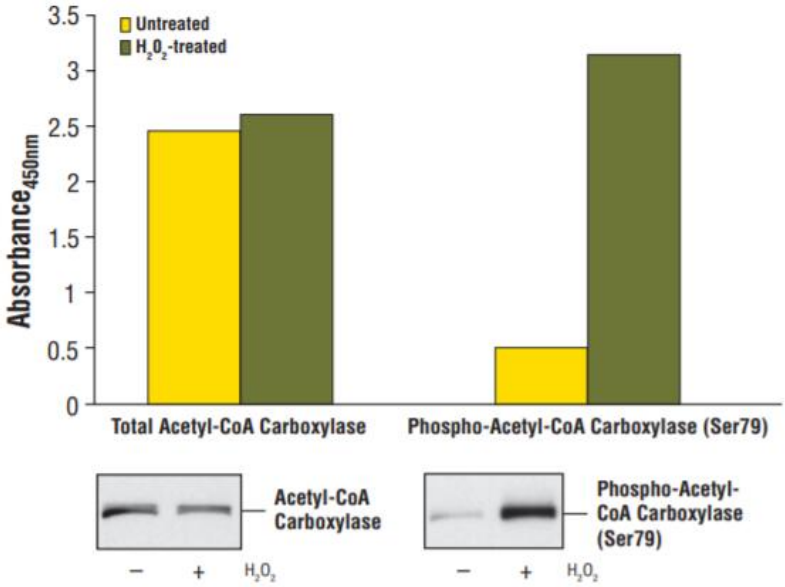

Figure 1. Treatment of Hep G2 cells with H2 O2 stimulates phosphorylation of ACC at Ser79, detected by the PhosphoACC (Ser79) Sandwich ELISA Kit, but does not affect the levels of total ACC detected by Total ACC Sandwich ELISA Kit. Hep G2 cells (80-90% confluent) were treated 10 mM hydrogen peroxide for 10 minutes and lysed with. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using Acetyl-CoA Carboxylase Rabbit mAb (left panel) or Phospho-Acetyl-CoA Carboxylase (Ser79) Antibody (right panel) are shown in the bottom figure.

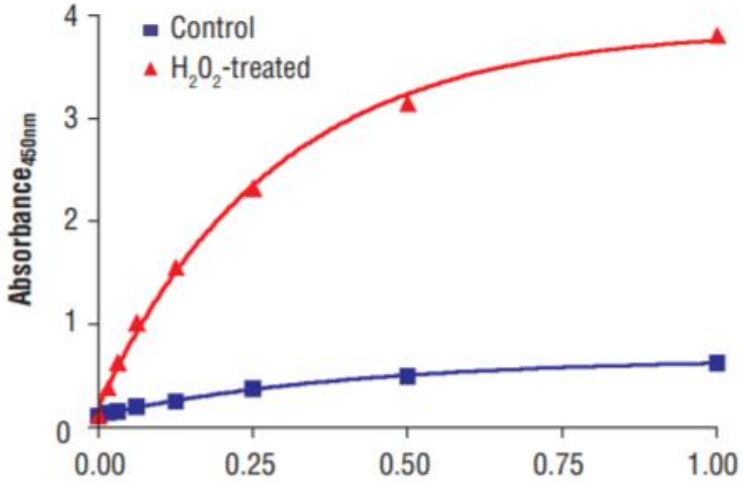

Figure 2. The relationship between the protein concentration of lysates from untreated and H2O2-treated Hep G2 cells and the absorbance at 450 nm using the Phospho-Acetyl-CoA Carboxylase (Ser79) Sandwich ELISA Kit is shown.

References

(1) Ruderman, N.B. et al. (1999) Am. J. Physiol. 276, E1-E18.

(2) Ha, J. et al. (1994) J. Biol. Chem. 269, 22162-22168.

(3) Abu-Elheiga, L. et al. (2001) Science 291, 2613-2616.

(4) Levert, K.L. et al. (2002) J. Biol. Chem. 277, 16347-16350.