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

Recomended Antibody Dilutions:
Western blotting 1:1000

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

U.S. Patent No. 5,675,063

Specification/Sensitivity: AceCS1 (D19C6) Rabbit mAb recognizes endogenous levels of total cytoplasmic acetyl-CoA synthetase. Phospho-Acetyl-CoA Carboxylase (Ser79) (D7D11) Rabbit mAb recognizes endogenous levels of ACC only when phosphorylated at Ser79; this antibody recognizes both ACCα and ACCβ. Acetyl-CoA Carboxylase (C83B10) Rabbit mAb recognizes endogenous levels of all isoforms of acetyl-CoA carboxylase protein. Phospho-ATP-Citrate Lyase (Ser455) Antibody recognizes endogenous levels of ATP-citrate lyase only when phosphorylated at Ser455. ACSL1 (D2H5) Rabbit mAb, ATP-Citrate Lyase Antibody, Fatty Acid Synthase (C20G5) Rabbit mAb, and Lipin 1 (D2W9G) Rabbit mAb recognize both ACCα and ACCβ. Acetyl-CoA Carboxylase α only when phosphorylated at Ser79; this antibody recognizes endogenous levels of total cytoplasmic acetyl-CoA carboxylase protein. Modification state-specific polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues 79 of human acetyl-CoA carboxylase and a synthetic phosphopeptide corresponding to residues surrounding Ala257 of human ACSL1 protein. Monoclonal long-chain acyl-CoA synthetase (ACSL) catalyzes the ligation of the fatty acid to CoA to form fatty acyl-CoA in a two-step reaction (4). ATP-citrate lyase (ACL) is a homotetramer that catalyzes the formation of acetyl-CoA and oxaloacetate (OAA) in the cytosol, which is the key step for the biosynthesis of fatty acids, cholesterol, and acetylocholine, as well as for gluconeogenesis (5). Phosphorylation of ACL at Ser455 abolishes the homotropic allosteric regulation by citrate and enhances the catalytic activity of the enzyme (6). Fatty acid synthase (FAS) catalyzes the synthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA (7). Lipin 1 plays a role in lipid metabolism in various tissues and cell types including liver, muscle, adipose tissues, and neuronal cell lines (8-10). It has dual functions at the molecular level: Lipin 1 serves as a transcriptional coactivator in the liver and a phosphatidate phosphatase in triglyceride and phospholipid biosynthesis pathways (11).

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. 20X Phosphate Buffered Saline (PBS): (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
2. 10X Tris Buffered Saline (TBS): (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
3. 1X SDS Sample Buffer: Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 3X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
4. 10X Tris-Glycine SDS Running Buffer: (#4050) To prepare 1 L 1X running buffer: add 100 ml 1X running buffer to 900 ml dH₂O, mix.
5. 10X Tris-Glycine Transfer Buffer: (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH₂O, mix.
6. 10X Tris Buffered Saline with Tween® 20 (TBST): (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
7. Nonfat Dry Milk: (#9999)
8. Blocking Buffer: 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. Wash Buffer: (#9997) 1X TBST
10. Bovine Serum Albumin (BSA): (#9998)
11. Primary Antibody Dilution Buffer: 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
13. Prestained Protein Marker, Broad Range (Premixed Format): (#7720)
14. Blotting Membrane and Paper: (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. Secondary Antibody Conjugated to HRP: anti-rabbit (#7074); anti-mouse (#7076)
16. Detection Reagents: Lumiglo® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately aspirate the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). NOTE: Loading of prestained molecular weight markers (#7720, 10 µl/tube) to verify electrophoresis and biotinylated protein ladder (#7727, 10 µl/tube) to determine molecular weights are recommended.
8. Electrophoresis to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml Lumiglo® (0.5 ml 20X Lumiglo® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.