#12589 Store at -20°C

Adipogenesis Marker Antibody Sampler Kit

1 Kit
(7 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Acetyl-CoA Carboxylase (C83B10) Rabbit mAb	3676	20 µl	280 kDa	Rabbit IgG
Adiponectin (C45B10) Rabbit mAb	2789	20 µl	27 kDa	Rabbit IgG
C/EBPα (D56F10) XP® Rabbit mAb	8178	20 µl	42, 28 kDa	Rabbit IgG
FABP4 Antibody	2120	20 µl	15 kDa	Rabbit
Fatty Acid Synthase (C20G5) Rabbit mAb	3180	20 µl	273 kDa	Rabbit IgG
Perilipin (D1D8) XP® Rabbit mAb	9349	20 µl	62 kDa	Rabbit IgG
PPARγ (C26H12) Rabbit mAb	2435	20 µl	53, 57 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Adipogenesis Marker Antibody Sampler Kit provides an economical means to evaluate proteins involved in the regulation of adipogenesis. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Adipocytes are the primary cellular component of adipose tissue and play a key role in the storage of triacylglycerol. Adipogenesis is the cellular process where preadipocytes differentiate into adipocytes.

Fatty acid binding proteins (FABPs) act as cytoplasmic lipid chaperones by binding fatty acids and lipids for transport to various cellular pathways (1,2). The predominant fatty acid binding protein found in adipocytes is FABP4.

Adiponectin is an adipokine expressed exclusively in brown and white adipocytes and is secreted into the blood. It exists in three major forms: a low molecular weight trimer, a medium molecular weight hexamer and a high molecular weight multimer (3). Decreased adiponectin levels are seen in obese and insulin-resistant mice and humans (4), suggesting that this adipokine is critical for maintenance of insulin sensitivity.

Peroxisome proliferator-activated receptor γ (PPARγ) is a transcriptional activator preferentially expressed in adipocytes, vascular smooth muscle cells, and macrophages (5,6).

Acetyl-CoA carboxylase (ACC) is a key fatty acid biosynthesis and oxidation enzyme that is responsible for the carboxylation of acetyl-CoA to malonyl-CoA, (7). Phosphorylation of acetyl-CoA carboxylase by AMPK at Ser79 or by PKA at Ser1200 inhibits ACC enzymatic activity (8). ACC is a potential target of anti-obesity drugs (9,10).

CCAAT/enhancer-binding proteins (C/EBPs) transcription factors are critical for cellular differentiation, terminal function, and the inflammatory response (11). Phosphorylation of C/EBPα at Thr222, Thr226, and Ser230 by GSK-3 may be required for adipogenesis (12).

Perilipin localizes to the periphery of lipid droplets and serves as a protective coating against lipases. Evidence suggests that PKA regulates lipolysis by phosphorylating perilipin (13-17), resulting in a conformational change that exposes lipid droplets to endogenous, hormone-sensitive lipases (14). Hence, perilipin plays a pivotal role in lipid storage (14,17).

Fatty acid synthase (FASN) catalyzes the synthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA. FASN is active as a homodimer with seven different catalytic activities and produces lipids in the liver for export to metabolically active tissues or storage in adipose tissue. In most other human tissues, FASN is minimally expressed since they rely on circulating fatty acids for new structural lipid synthesis (18).

Specificity/Sensitivity: Each antibody recognizes endogenous total levels of its specific target protein. The Adiponectin (C45B10) Rabbit mAb detects endogenous levels of total adiponectin protein monomer. It will not detect higher molecular weight forms of adiponectin. The Acetyl-CoA Carboxylase (C83B10) Rabbit mAb detects endogenous levels of all isoforms of acetyl-CoA carboxylase protein. The FABP4 Antibody may cross react with other FABP family members.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser523 of human acetyl-CoA carboxylase α1, to human adiponectin, to the sequence of mouse FABP4, to residues surrounding Gly46 of human fatty acid synthase, to residues surrounding Ile419 of human perilipin/perilipin-1 protein, to residues surrounding Ala176 of human C/EBPα protein, or to residues surrounding Asp69 of human PPARγ.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:

Western blotting	1:1000

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

Background References:

(1) Tuncman, G. et al. (2006) *Proc Natl Acad Sci USA* 103, 6970-5.
(2) Haunerland, N.H. and Spener, F. (2004) *Prog Lipid Res* 43, 328-49.
(3) Kadowaki, T. et al. (2006) *J Clin Invest* 116, 1784-92.
(4) Hu, E. et al. (1996) *J Biol Chem* 271, 10697-703.
(5) Tontonoz, P. et al. (1995) *Curr Opin Genet Dev* 5, 571-6.
(6) Rosen, E.D. et al. (1999) *Mol Cell* 4, 611-7.
(7) Castle, J.C. et al. (2009) *PLoS One* 4, e4369.
(8) Ha, J. et al. (1994) *J Biol Chem* 269, 22162-8.
(9) Abu-Elheiga, L. et al. (2001) *Science* 291, 2613-6.
(10) Levert, K.L. et al. (2002) *J Biol Chem* 277, 16347-50.
(11) Lekstrom-Himes, J. and Xanthopoulos, K.G. (1998) *J Biol Chem* 273, 28545-8.
(12) Ross, S.E. et al. (1999) *Mol Cell Biol* 19, 8433-41.
(13) Greenberg, A.S. et al. (1991) *J Biol Chem* 266, 11341-6.
(14) Brasaemle, D.L. (2007) *J Lipid Res* 48, 2547-59.
(15) Ducharme, N.A. and Bickel, P.E. (2008) *Endocrinology* 149, 942-9.
(16) Egan, J.J. et al. (1990) *J Biol Chem* 265, 18769-75.
(17) Brasaemle, D.L. et al. (2009) *Mol Cell Biochem* 326, 15-21.
(18) Katsurada, A. et al. (1990) *Eur J Biochem* 190, 427-33.

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected
Species enclosed in parentheses are predicted to react based on 100% homology.

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 dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, 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 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, 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 dH_2O, 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 dH_2O, 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.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
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 Reagent:** 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 scrape 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/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) 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.