#8660 Store at -20°C

PPARγ Regulated Fatty Acid Metabolism Antibody Sampler Kit

☑ 1 Kit
(7 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
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info@cellsignal.com
Web ■ www.cellsignal.com

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-AMPKα (Thr172) (40H9) Rabbit mAb	2535	20 µl	62 kDa	Rabbit IgG
AMPKα (D5A2) Rabbit mAb	5831	20 µl	62 kDa	Rabbit IgG
CBP (D6C5) Rabbit mAb	7389	20 µl	300 kDa	Rabbit IgG
GCN5L2 (C26A10) Rabbit mAb	3305	20 µl	94 kDa	Rabbit IgG
PPARγ (C26H12) Rabbit mAb	2435	20 µl	53, 57 kDa	Rabbit IgG
SirT1 (C14H4) Rabbit mAb	2496	20 µl	120 kDa	Rabbit IgG
RXRα (D6H10) Rabbit mAb	3085	20 µl	53 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.

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 antibody.*

Recommended Antibody Dilutions:
Western blotting 1:1000

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

Description: PPARγ Regulated Fatty Acid Metabolism Antibody Sampler Kit provides an economical means to evaluate PPARγ and related proteins involved in lipid metabolism. This kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: 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) (1). 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 phosphorylates AMPKα at Thr172 in the activation loop, and this phosphorylation is required for AMPK activation (2-4). 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 (5).

CBP (CREB-binding protein) is a transcriptional co-activator that associates with PPARγ (6,7). CBP also contains histone acetyltransferase (HAT) activity, allowing it to acetylate histones and other proteins (7).

General Control of Amino Acid Synthesis Yeast Homolog Like 2 (GCN5L2) is a transcription adaptor protein and a histone acetyltransferase (HAT) that functions as the catalytic subunit of the STAGA and TFTC transcription coactivator complexes (8). GCN5L2 is 73% homologous to the p300/CBP-associated factor PCAF, another HAT protein found in similar complexes (9). GCN5L2 acetylates non-histone proteins such as the transcription co-activator PGC1-α (10).

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the ligand-activated nuclear receptor superfamily and functions as a transcriptional activator (11). PPARγ is preferentially expressed in adipocytes as well as in vascular smooth muscle cells and macrophage (12).

The Silent Information Regulator (SIR2) family of genes is a highly conserved group of genes that encode nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases, also known as class III histone deacetylases (13). SirT1, the mammalian ortholog of Sir2, is a nuclear protein implicated in the regulation of many cellular processes, including apoptosis, cellular senescence, endocrine signaling, glucose homeostasis, aging, and longevity. Targets of SirT1 include PPARγ (14), and the PPARγ coactivator-1α (PGC-1α) protein (15). Deacetylation of PPARγ and PGC-1α regulates the gluconeogenic/glycolytic pathways in the liver and fat mobilization in white adipocytes in response to fasting (14,15).

The human retinoid X receptors (RXRs) are type-II nuclear hormone receptors encoded by three distinct genes (RXRα, RXRβ, and RXRγ) and bind selectively and with high affinity to the vitamin A derivative, 9-cis-retinoic acid. Nuclear RXRs form heterodimers with PPAR to help regulate transcription during lipid metabolism (16).

Specificity/Sensitivity: Phospho-AMPKα (Thr172) (40H9) Rabbit mAb detects endogenous AMPKα only when phosphorylated at Thr172. Phospho-AMPKα (Thr172) (40H9) Rabbit mAb detects both α1 and α2 isoforms of the catalytic subunit, but does not detect the regulatory β or γ subunits. AMPKα (D5A2) Rabbit mAb, CBP (D6C5) Rabbit mAb, GCN5L2 (C26A10) Rabbit mAb, PPARγ (C26H12) Rabbit mAb, SirT1 (C14H4) Rabbit mAb, and RXRα (D6H10) Rabbit mAb all detect endogenous levels of their respective total proteins.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr172 of human AMPKα protein or with a synthetic peptide corresponding to the respective sequences of human AMPKα, CBP, GCN5L2, PPARγ, SirT1 and RXRα protein.

Background References:

(1) Carling, D. (2004) *Trends Biochem Sci* 29, 18-24.
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(3) Lizcano, J.M. et al. (2004) *EMBO J* 23, 833-43.
(4) Shaw, R.J. et al. (2004) *Proc Natl Acad Sci USA* 101, 3329-35.
(5) Hardie, D.G. (2004) *J Cell Sci* 117, 5479-87.
(6) Goodman, R.H. and Smolik, S. (2000) *Genes Dev* 14, 1553-77.
(7) Chan, H.M. and La Thangue, N.B. (2001) *J Cell Sci* 114, 2363-73.
(8) Candau, R. et al. (1996) *Mol Cell Biol* 16, 593-602.
(9) Yang, X.J. et al. (1996) *Nature* 382, 319-24.
(10) Lerin, C. et al. (2006) *Cell Metab* 3, 429-38.
(11) Tontonoz, P. et al. (1995) *Curr Opin Genet Dev* 5, 571-6.
(12) Rosen, E.D. et al. (1999) *Mol Cell* 4, 611-7.
(13) Guarente, L. (1999) *Nat Genet* 23, 281-5.
(14) Picard, F. et al. (2004) *Nature* 429, 771-6.
(15) Rodgers, J.T. et al. (2005) *Nature* 434, 113-8.
(16) Gronemeyer, H. et al. (2004) *Nat Rev Drug Discov* 3, 950-64.

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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 **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#8660

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.