#12814 Store at -20°C

C/EBP Antibody Sampler Kit

☑ 1 Kit
(7 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

New 06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-C/EBPα (Ser21) Antibody	2841	20 µl	45 kDa	Rabbit IgG
Phospho-C/EBPα (Thr222/226) Antibody	2844	20 µl	30, 42, 45 kDa	Rabbit IgG
C/EBPα (D56F10) XP® Rabbit mAb	8178	20 µl	42, 28 kDa	Rabbit IgG
Phospho-C/EBPβ (Thr235) Antibody	3084	20 µl	19, 36, 38 kDa	Rabbit IgG
C/EBPβ (LAP) Antibody	3087	20 µl	35-38 kDa 45-49 kDa	Rabbit IgG
C/EBPδ Antibody	2318	20 µl	29 kDa	Rabbit IgG
CHOP (D46F1) Rabbit mAb	5554	20 µl	27 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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

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

Description: The C/EBP Antibody Sampler Kit provides an economical means of evaluating the C/EBP family of transcription factors and several phosphorylation sites that are involved in its activation. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: CCAAT/enhancer-binding proteins (C/EBPs) are transcription factors critical for cellular differentiation, terminal function, and inflammatory response. Six characterized family members (C/EBPα, β, δ, γ, ε, and ζ) are distributed in a variety of tissues (1). Translation from alternative start codons results in two C/EBPα isoforms (p42 and p30) that are strong transcriptional activators (2). Research studies indicate that insulin and insulin-like growth factor-I stimulate C/EBPα dephosphorylation, which may play a key role in insulin-induced repression of *GLUT4* transcription (3). Phosphorylation of C/EBPα at Thr222, Thr226, and Ser230 by GSK-3 may be required for adipogenesis (4).

The two forms of C/EBPβ, 38 kDa liver activating protein (LAP) and the 20 kDa liver inhibitory protein (LIP), may result from alternative translation. The 38 kDa LAP protein is a transcriptional activator while LIP may inhibit C/EBPβ transcriptional activity (5).

Phosphorylation of C/EBPβ at distinct sites stimulates its transcriptional activity (6-8). Phosphorylation at the rat-specific site Ser105 in C/EBPβ appears essential for C/EBPβ activation in rat (9). C/EBPδ protein is highly expressed in adipose tissue, lung, and intestine (10). Increased expression of C/EBPδ mRNA levels during adipogenesis suggests that C/EBPδ plays an important role in positively regulating adipogenesis (10,11). C/EBPδ is expressed in the mammalian nervous system and plays an important role in long-term memory (10,12). CHOP is a C/EBP-homologous protein that inhibits C/EBP and LAP in a dominant-negative manner (13). CHOP expression is induced by cellular stresses, including starvation; induced CHOP suppresses cell cycle progression from G1 to S phase (14). During ER stress, the level of CHOP expression is elevated and CHOP functions to mediate programmed cell death (15).

Specificity/Sensitivity: Unless otherwise indicated, each antibody will recognize endogenous total levels of their target protein. Each activation state antibody recognizes the phosphorylated form of its target. Phospho-C/EBPβ (Thr235) Antibody recognizes endogenous levels of human liver activating protein (LAP) only when phosphorylated at Thr235, mouse and rat LAP only when phosphorylated at Thr188, and liver inhibitory protein (LIP) only when phosphorylated at Thr37. The C/EBPβ (LAP) Antibody detects endogenous levels of total C/EBPβ, the p38 and p36 LAPs, but not the p20 LIP.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser21 of human C/EBPα, Thr222/226 of mouse C/EBPα, or Thr235 of human C/EBPβ. Polyclonal antibodies are also produced by immunizing animals with a synthetic peptide corresponding to the amino-terminal sequence of human C/EBPβ or the sequence of mouse C/EBPδ. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ala176 of human C/EBPα protein or to residues surrounding Leu159 of human CHOP protein.

Background References:

(1) Lekstrom-Himes, J. and Xanthopoulos, K.G. (1998) *J Biol Chem* 273, 28545-8.
(2) Lin, F.T. et al. (1993) *Proc Natl Acad Sci U S A* 90, 9606-10.
(3) Hemati, N. et al. (1997) *J Biol Chem* 272, 25913-9.
(4) Ross, S.E. et al. (1999) *Mol Cell Biol* 19, 8433-41.
(5) Calkhoven, C.F. et al. (2000) *Genes Dev* 14, 1920-32.
(6) Wegner, M. et al. (1992) *Science* 256, 370-3.
(7) Trautwein, C. et al. (1993) *Nature* 364, 544-7.
(8) Nakajima, T. et al. (1993) *Proc Natl Acad Sci USA* 90, 2207-11.
(9) Buck, M. et al. (1999) *Mol Cell* 4, 1087-92.
(10) Ramji, D.P. and Foka, P. (2002) *Biochem J* 365, 561-75.
(11) Cao, Z. et al. (1991) *Genes Dev* 5, 1538-52.
(12) Taubenfeld, S.M. et al. (2001) *J Neurosci* 21, 84-91.
(13) Ron, D. and Habener, J.F. (1992) *Genes Dev* 6, 439-53.
(14) Barone, M.V. et al. (1994) *Genes Dev* 8, 453-64.
(15) Zinszner, H. et al. (1998) *Genes Dev* 12, 982-95.

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.

#12814

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.