

#2841 Store at -20°C

# Phospho-C/EBP $\alpha$ (Ser21) Antibody

100  $\mu$ l  
 (10 Western mini-blot)



**Orders** ■ 877-616-CELL (2355)  
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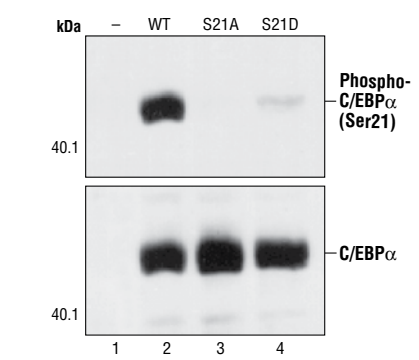
rev. 02/13/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, (R, B)	45 kDa	Rabbit

**Background:** CCAAT/enhancer-binding proteins (C/EBPs) are a family of transcription factors that are critical for cellular differentiation, terminal function and inflammatory response (1). Six members of the family have been characterized (C/EBP $\alpha$ ,  $\beta$ ,  $\delta$ ,  $\gamma$ ,  $\epsilon$  and  $\zeta$ ) and are distributed in a variety of tissues (1). Translation from alternative start codons results in two isoforms of C/EBP $\alpha$  (p42 and p30), which are both strong transcriptional activators (2). It has been reported that insulin and insulin-like growth factor-1 stimulate the dephosphorylation of C/EBP $\alpha$ , which may play a key role in insulin induced repression of GLUT4 transcription (3). Phosphorylation of C/EBP $\alpha$  at Thr222, Thr226 and Ser230 by GSK3 seems to be required for adipogenesis (4).

**Specificity/Sensitivity:** Phospho-C/EBP $\alpha$  (Ser21) Antibody detects endogenous levels of C/EBP $\alpha$  only when phosphorylated at Ser21. This antibody does not cross-react with other phosphorylated C/EBP isoforms.

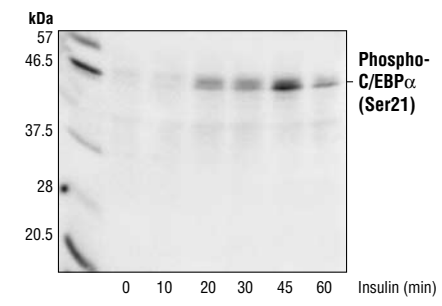


Western blot analysis of extracts of COS cells untransfected (lane 1), or transfected with wild-type mouse C/EBP $\alpha$  (lane 2), S21A (lane 3), and S21D (lane 4) mutants, using Phospho-C/EBP $\alpha$  (Ser21) Antibody (upper) or C/EBP $\alpha$  Antibody (lower). (Provided by Dr. Hanna Radomska, Beth Israel Deaconess Medical Center, Boston, MA).

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser21 of human C/EBP $\alpha$ . Antibodies are purified by protein A and peptide affinity chromatography.

**Selected Application References:**  
 Ross, S.E. et al. (2004) Phosphorylation of C/EBP $\alpha$  inhibits granulopoiesis. Phosphorylation of C/EBP $\alpha$  inhibits granulopoiesis. *Mol. Cell. Biol.* 24, 675–686. Application: W.

- Background References:**
- (1) Lekstrom-Hims, J. and Xanthopoulos, K.G. (1998) *J. Biol. Chem.* 273, 28545–28548.
  - (2) Lin, F. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 9606–9610.
  - (3) Hemati, N. et al. (1997) *J. Biol. Chem.* 272, 25913–25919.
  - (4) Ross, S.E. et al. (1999) *Mol. Cell. Biol.* 19, 8433–8441.
  - (5) Radomska, H.S. et al. (2006) *J Exp Med* 203, 371–81.



Western blot analysis of extracts from mouse adipocytes treated with insulin for the indicated times using Phospho-C/EBP $\alpha$  (Ser21) Antibody.

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Entrez-Gene ID** #1050  
**Swiss-Prot Acc.** #P49715  
**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA and 50% glycerol. Store at -20°C. *Do not aliquot the antibody.*

**\*Species cross-reactivity is determined by Western blot.**

**Recommended Antibody Dilutions:**  
 Western Blotting 1:1000

- Companion Products:**
- Phospho-C/EBP $\beta$  (Thr235) Antibody #3084
  - Phospho-C/EBP $\alpha$  (Thr222/226) Antibody #2844
  - C/EBP $\alpha$  Antibody #2295
  - C/EBP $\beta$  Antibody #3082
  - Phospho-C/EBP $\beta$  (Ser105) Antibody (Rat Specific) #3081
  - C/EBP $\delta$  Antibody #2318
  - C/EBP $\alpha$  (p42) Antibody #2843
  - C/EBP $\beta$  (LAP) Antibody #3087
  - Phototope<sup>®</sup>-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
  - Anti-rabbit IgG, HRP-linked Antibody #7074
  - Prestained Protein Marker, Broad Range (Premixed Format) #7720
  - Biotinylated Protein Ladder Detection Pack #7727
  - 20X LumiGLO<sup>®</sup> Reagent and 20X Peroxide #7003

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.