

# Phospho-C/EBP $\beta$ (Thr235) Antibody

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

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

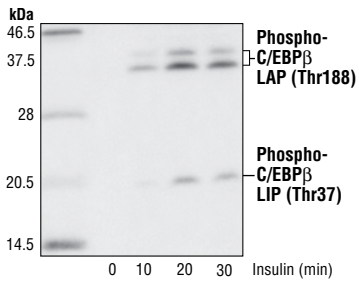
Applications	Species Cross-Reactivity	Molecular Wt.	Source
W	H, M, (B)	19 k Da LIP 36 kDa LAP 38 kDa LAP.	Rabbit

**Background:** CCAAT/enhancer-binding proteins (C/EBPs) are a family of transcription factors critical for cellular differentiation, terminal functions 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). There are two forms of C/EBP $\beta$ , the 38 kDa liver activating protein (LAP) and the 20 kDa liver inhibitory protein (LIP) which may be products of alternative translation. The 38 kDa LAP protein is a transcriptional activator while LIP may act as an inhibitor of C/EBP $\beta$  transcriptional activity (2). Phosphorylation of C/EBP $\beta$  at distinct sites stimulates its transcriptional activity (3-5). Phosphorylation at serine 105 of rat C/EBP $\beta$ , a unique site only present in the rat sequence, seems essential for rat C/EBP $\beta$  activation (6).

**Specificity/Sensitivity:** Phospho-C/EBP $\beta$  (Thr235) Antibody detects endogenous levels of human LAP only when phosphorylated at Thr235, mouse and rat LAP only when phosphorylated at Thr188, and LIP only when phosphorylated at Thr37. This antibody does not cross-react with other phosphorylated C/EBPs.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding threonine 235 of human C/EBP $\beta$ . Antibodies are purified by protein A and peptide affinity chromatography.

**Selected Application References:**  
 Pwien-Pilipuk, G. et al. (2002). Dual regulation of phosphorylation and dephosphorylation of C/EBP $\beta$  modulate its transcriptional activation and DNA binding in response to growth hormone. *J. Biol. Chem.* 277, 44557-44565.  
 Application: W.



Western blot analysis of extracts from adipocytes (differentiated 3T3-L1) treated with insulin for the indicated times, using Phospho-C/EBP $\beta$  (Thr235) Antibody.

**Background References:**

- (1) Lekstrom-Himes, J. and Xanthopoulos, K.G. (1998) *J. Biol. Chem.* 273, 28545-28548.
- (2) Calkhoven, C.F. et al. (2000) *Genes Dev* 14, 1920-1932.
- (3) Wegner, M. et al. (1992) *Science* 256, 370-373.
- (4) Trautwein, C. et al. (1993) *Nature* 364, 544-547.
- (5) Nakajima, T. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 2207-2211.
- (6) Buck, M. et al. (1999) *Mol. Cell* 4, 1087-1092.

**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.

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

**Companion Products:**  
 Phospho-C/EBP $\alpha$  (Ser21) Antibody #2841  
 C/EBP $\beta$  (LAP) Antibody #3087  
 Phospho-C/EBP $\beta$  (Ser105) Antibody (Rat Specific) #3081  
 C/EBP $\beta$  Antibody #3082  
 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

**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.**

## Western Immunoblotting Protocol (Primary Ab 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.