#8686 Store at -20°C

Lysine Acetyltransferase Antibody Sampler Kit

☑ 1 Kit
(4 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Entrez-Gene ID #1387, 2648, 8850
UniProt ID #Q92793, Q92830, Q92831

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Acetyl-CBP (Lys1535)/p300 (Lys1499) Antibody	4771	20 µl	300 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
PCAF (C14G9) Rabbit mAb	3378	20 µl	93 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 136 mM NaCl, 2.6 mM KCl, 12 mM sodium phosphate (pH 7.4) dibasic, 2 mg/ml BSA, and 50% glycerol. *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: The Lysine Acetyltransferase Antibody Sampler Kit provides an economical means to examine several lysine acetyltrasferases, including: Acetyl-CBP, CBP, GCN5L2, and PCAF. The kit includes enough antibody to perform two western blot experiments with each per primary antibody.

Background: CREB-binding protein (CBP) and p300 are highly conserved and functionally related transcriptional co-activators that associate with transcriptional regulators and signaling molecules, integrating multiple signal transduction pathways with the transcriptional machinery (1,2). CBP/p300 also contain histone acetyltransferase (HAT) activity, allowing them to acetylate histones and other proteins (2). The role of acetylation of CBP/p300 is of particular interest (2,3). Acetylation of p300 at Lys1499 has been demonstrated to enhance its HAT activity and affect a wide variety of signaling events (4). p300/CBP-associated factor (PCAF), also known as lysine acetyl-transferase 2B (KAT2B) (5), and General Control of Amino Acid Synthesis Yeast Homolog Like 2 (GCN5L2) (6) are transcriptional adaptor proteins in addition to HATs. PCAF functions as the catalytic subunit of the PCAF transcriptional co-activator complex (5). GCN5L2 functions as the catalytic subunit of the STAGA and TFTC transcription coactivator complexes (6). PCAF and GCN5L2 acetylate histone H3 at Lys14 and histone H4 at Lys8, both of which contribute to gene activation by modulating chromatin structure and recruiting additional co-activator proteins that contain acetyl-lysine binding bromo-domains (7,8). PCAF also acetylates non-histone proteins including transcriptional activators (p53, E2F1, MyoD) and general transcription factors (TFIIEβ and TFIIF) (9-12). GCN5L2 also acetylates non-histone proteins such as transcription activators (TAT, c-Myb) (13,14), transcription co-activators (PGC1-α) (15), and nuclear receptors (Steroidogenic Factor 1) (16). Acetylation of these proteins regulates their nuclear localization, protein stability, DNA binding, and co-activator association (13-16).

Specificity/Sensitivity: Each antibody in the Lysine Acetyltransferase Antibody Sampler Kit recognizes endogenous levels of respective target protein. The antibodies do not cross-react with other family members.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the amino terminus of human CBP protein, human GCN5L2 protein, or human PCAF protein. Polyclonal antibodies are produced by immunizing animals with a synthetic acetylated peptide corresponding to residues surrounding Lys1535 of human CBP. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Goodman, R.H. and Smolik, S. (2000) *Genes Dev* 14, 1553-77.
(2) Chan, H.M. and La Thangue, N.B. (2001) *J Cell Sci* 114, 2363-73.
(3) Yuan, L.W. and Giordano, A. (2002) *Oncogene* 21, 2253-60.
(4) Thompson, P.R. et al. (2004) *Nat Struct Mol Biol* 11, 308-15.
(5) Nagy, Z. and Tora, L. (2007) *Oncogene* 26, 5341-57.
(6) Candau, R. et al. (1996) *Mol Cell Biol* 16, 593-602.
(7) Schiltz, R.L. et al. (1999) *J Biol Chem* 274, 1189-92.
(8) Grant, P.A. et al. (1999) *J Biol Chem* 274, 5895-900.
(9) Bannister, A.J. and Miska, E.A. (2000) *Cell Mol Life Sci* 57, 1184-92.
(10) Liu, L. et al. (1999) *Mol Cell Biol* 19, 1202-9.
(11) Sartorelli, V. et al. (1999) *Mol Cell* 4, 725-34.
(12) Imhof, A. et al. (1997) *Curr Biol* 7, 689-92.
(13) Kiernan, R.E. et al. (1999) *EMBO J* 18, 6106-18.
(14) Tomita, A. et al. (2000) *Oncogene* 19, 444-51.
(15) Lerin, C. et al. (2006) *Cell Metab* 3, 429-38.
(16) Jacob, A.L. et al. (2001) *J Biol Chem* 276, 37659-64.

U.S. Patent No. 5,675,063

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.

#8686

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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **Phototope®-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® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

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 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

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

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. 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® incubation and declines over the following 2 hours.