

PathScan® Acetylated Histone H3 Sandwich ELISA Antibody Pair

✓ 1 Kit
(4 X 96 assays)



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New 11/07

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

Entrez-Gene ID #8352
Swiss-Prot Acc. #P68431

Species Cross-Reactivity: H, M, Mk

Description: CST's PathScan® Acetylated Histone H3 Sandwich ELISA Antibody Pair is being offered as an alternative to our PathScan® Acetylated Histone H3 Sandwich ELISA Kit #7232. Capture and Detection antibodies (100X stocks) and HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are provided for performing 4 x 96 well ELISAs. The Histone Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added, followed by an Acetylated-Lysine Detection Antibody and HRP-conjugated Anti-Mouse IgG antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance at 450 nm is proportional to the quantity of Acetylated Histone H3 protein.

*Antibodies in this kit are custom formulations specific to the kit

Reagents not supplied:

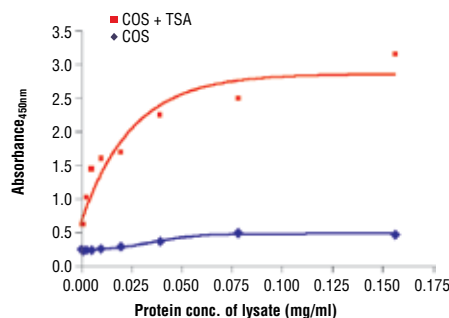
- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween -20 (PBST-20X) #9809
- Cell Lysis Buffer (10X) #9803
- TMB Substrate #7004
- STOP Solution #7002
- Blocking Buffer- PBS+0.05% Tween-20, 1% BSA
- 96 Well Microplates**
- Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Note: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

Background: Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of four core histone proteins (H2A, H2B, H3 and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination (2-5). These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, on gene expression (6). In most species, histone H2B is pri-

Products Included	Volume	Cap Color	Storage
Total Histone H3 Capture Antibody (100X)	0.4 ml	Pink	4°C
Acetylated-Lysine Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-Mouse IgG, HRP-Linked Antibody (1000X)	0.04 ml	Yellow	-20°C



The relationship between lysate protein concentration from untreated and TSA treated COS cells and the absorbance at 450 nm using PathScan® Acetylated Histone H3 Sandwich ELISA Antibody Pair #7209 is shown. COS cells were lysed following overnight treatment with TSA at 37°C.

marily acetylated at Lys5, 12, 15 and 20 (4,7). Histone H3 is primarily acetylated at Lys9, 14, 18 and 23 (2,3). Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms (2,3). Phosphorylation at Ser10, Ser28 and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (8-10). Phosphorylation of Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation of H3 Thr3 in prophase and its dephosphorylation during anaphase (11).

Storage: Histone H3 Capture Antibody and Acetylated-Lysine Detection Antibody are stored at 4°C. Anti-Mouse IgG, HRP-Linked Antibody is stored at -20°C.

Companion Products:

- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809
- BSA #9998
- TMB Substrate #7004
- STOP Solution #7002
- PathScan® Acetylated Histone H3 Sandwich ELISA Kit #7232
- Histone H3 Antibody #9715
- Histone H3 (3H1) Rabbit mAb #9717
- Acetyl-Histone H3 (Lys18) Antibody #9675
- Acetyl-Histone H3 (Lys23) Antibody #9674
- Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb #9649
- Acetyl-Histone H3 (Lys9) Antibody #9671
- Acetyl-Histone H3 (Lys9/Lys14) Antibody #9677
- Cell Lysis Buffer (10X) #9803
- Anti-mouse IgG, HRP-linked Antibody #7076

Background References:

- (1) Workman, J.L. and Kingston, R.E. (1998) *Annu. Rev. Biochem.* 67, 545-579.
- (2) Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637-17641.
- (3) Strahl, B.D. and Allis, C.D. (2000) *Nature* 403, 41-45.
- (4) Cheung, P. et al. (2000) *Cell* 103, 263-271.
- (5) Bernstein, B.E. and Schreiber, S.L. (2002) *Chem. Biol.* 9, 1167-1173.
- (6) Jaskelioff, M. and Peterson, C.L. (2003) *Nat. Cell Biol.* 5, 395-399.
- (7) Thorne, A.W. et al. (1990) *Eur. J. Biochem.* 193, 701-713.
- (8) Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348-360.
- (9) Goto, H. et al. (1999) *J. Biol. Chem.* 274, 25543-25549.
- (10) Preuss, U. et al. (2003) *Nucleic Acids Res.* 31, 878-885.
- (11) Dai, J. et al. (2005) *Genes Dev.* 19, 472-488.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIATM

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

- Coating Buffer:** 1X PBS, (20X PBS #9808)
3.2 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 1.3 mM KCl, 135 mM NaCl, pH 7.4
- Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
- 1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #9803)
20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA),
1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA),
1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate,
1 mM Na₃VO₄, 1 μg/ml leupeptin.
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

- Rinse microplate with dH₂O. Add 200 μl of dH₂O and discard liquid. Blot on paper towel to make sure wells are dry.
- Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 μl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 μl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
- After overnight coating, gently uncover plate and wash wells:**
 - Discard plate contents into a receptacle.
 - Wash 4 times with Wash Buffer, 200 μl each time for each well. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 - Clean the underside of all wells with a lint-free tissue.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

- Aspirate media, treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm²) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

D Test Procedure

- Lysates can be used undiluted or diluted in Blocking Buffer. 100 μl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
- Wash plate as in Coating Procedure, Step 3.
- Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 μl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover plate and incubate at 37°C for 1 hour.
- Plate is washed as in Coating Procedure, Step 3.
- Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 μl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover and incubate at 37°C for 30 minutes.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.