

# PathScan® Acetyl-Histone H2A Sandwich ELISA Kit

✓ 1 Kit  
(96 assays)

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

## Species Cross-Reactivity: H, M, Mk

**Introduction:** CST's PathScan® Acetyl-Histone H2A Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of acetylated lysines on Histone H2A. A Histone H2A Antibody has been coated onto the microwells. After incubation with cell lysates, Histone H2A is captured by the coated antibody. Following extensive washing, Acetylated-Lysine Mouse mAb (Ac-K-103) #9681\* is added to detect the acetylated lysines on the Histone H2A protein. Anti-mouse IgG, HRP linked Antibody #7076 is then used to recognize the bound detection antibody. HRP substrate, TMB is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of acetylated Histone H2A.

\* Antibodies in kit are custom formulations specific to kit.

## Companion Products:

Histone H2A Antibody II #2578

Acetylated-Lysine Mouse mAb (Ac-K-103) #9681

PathScan® Acetylated Histone H3 Sandwich ELISA Kit #7232

PathScan® Acetylated Histone H4 Sandwich ELISA Kit #7238

PathScan® Acetylated p53 Sandwich ELISA Kit #7236

PathScan® Acetylated Histone H2B Sandwich ELISA kit #7178

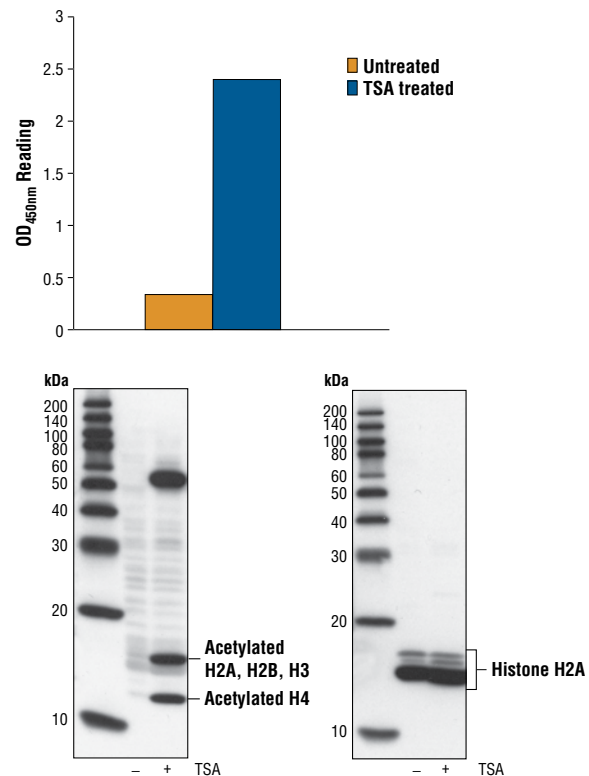
**Specificity/Sensitivity:** CST's PathScan® Acetyl-Histone H2A Sandwich ELISA Kit detects endogenous levels of Acetylated Histone H2A. Using this Sandwich ELISA Kit #7233, acetylated lysines on Histone H2A are detected when treated with TSA in Jurkat cells. However, the levels of Histone H2A remain unchanged, as shown by Western analysis using the Histone H2A Antibody #2578 (figure 1). COS and NIH 3T3 cells treated with TSA show similar results (data not shown).

Figure 1: Treatment of Jurkat cells with TSA causes accumulation of acetylation on Histone H2A, detected by Sandwich ELISA kit, #7233, but does not affect the level of total Histone H2A protein, detected by Western analysis.  $OD_{450nm}$  readings are shown in the top figure, while the corresponding Western blot using the Acetylated Lysine mouse mAb (Ac-K-103) #9681 (left panel) or Histone H2A Antibody #2578 (right panel), is shown in the bottom figure.

Products Included	Volume	Solution Color
Histone H2A Antibody Coated Microwells*	96 tests	
Acetylated-Lysine Detection Ab	11 ml	green
Anti-mouse IgG HRP-Linked Ab	11 ml	red
TMB Substrate	11 ml	colorless
STOP Solution	11 ml	colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	colorless
Sample Diluent	25 ml	blue
10X Cell Lysis Buffer #9803**	15 ml	yellowish

\* 12 8-well modules -Each module is designed to break apart for 8 tests.

\*\*Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).





**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 primarily acetylated at lysines 5, 12, 15 and 20 (4,7). Histone H3 is primarily acetylated at lysines 9, 14, 18 and 23 (2,3). Acetylation 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 (8,9,10). 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).

#### Background References:

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- (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.
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- (10) Preuss, U. et al. (2003) *Nucleic Acids Res.* 31, 878–885.
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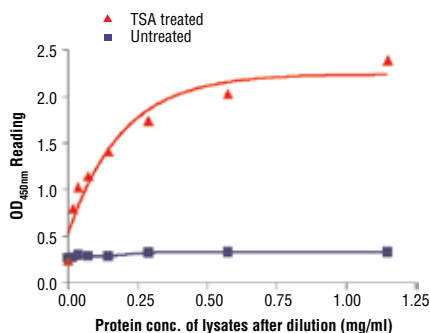


Figure 2: The relationship between protein concentration of lysates from untreated and TSA treated Jurkat cells and kit assay optical density readings. Jurkat cells were treated with TSA (0.4  $\mu$ M overnight).

## Sandwich ELISA Protocol

### A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #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 Na<sub>3</sub>VO<sub>4</sub>, 1 μg/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

### B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. 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.

### C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Add 100 μl of Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 μl of cell lysate into the tube and vortex for a few seconds. Generally, sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction. Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results. However, dilution factors need to be titrated when specific cell lysates are used.

3. Add 100 μl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.
4. Gently remove the tape and wash wells:
  - a. Discard plate contents into a receptacle.
  - b. Wash 4 times with 1X Wash Buffer, 200 μl each time for each well.
  - c. 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.
  - d. Clean the underside of all wells with a lint-free tissue.
5. Add 100 μl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step 4.
7. Add 100 μl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.
8. Repeat wash procedure as in Step 4.
9. Add 100 μl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes at 25°C.
10. Add 100 μl of STOP Solution to each well. Shake gently for a few seconds.

**NOTE:** Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

11. Read results.
  - a. Visual Determination — Read within 30 minutes after adding STOP Solution.
  - b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.