

PathScan® Acetyl-Histone H2B Sandwich ELISA Kit

✓ 1 Kit
(96 assays)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

rev. 06/07/11

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Species Cross-Reactivity: H, Mk

Introduction: The PathScan® Acetyl-Histone H2B Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of acetylated lysines on Histone H2B. A Histone H2B Antibody has been coated onto the microwells. After incubation with cell lysates, Histone H2B is captured by the coated antibody. Following extensive washing, an Acetylated-Lysine Mouse mAb is added to detect the acetylated lysines on the Histone H2B 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 H2B.

* Antibodies in kit are custom formulations specific to kit.

Companion Products:

Histone H2B Antibody #2722

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

PathScan® Acetylated Histone H3 Sandwich ELISA Kit #7232

PathScan® Acetylated Histone H2A Sandwich ELISA Kit #7233

PathScan® Acetylated p53 Sandwich ELISA Kit #7236

PathScan® Acetylated Histone H4 Sandwich ELISA #7238

Specificity/Sensitivity: CST's PathScan® Acetyl-Histone H2B Sandwich ELISA Kit detects endogenous levels of Acetylated Histone H2B. Using this Sandwich ELISA Kit #7178, acetylated lysines on Histone H2B are detected when treated with TSA in Cos cells. However, the levels of total Histone H2B protein remain unchanged, as shown by Western blot analysis, using the Histone H2B Antibody #2722 (figure 1). Jurkat cells treated with TSA show similar results (data not shown).

Products Included	Volume	Solution Color
Histone H2B 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).

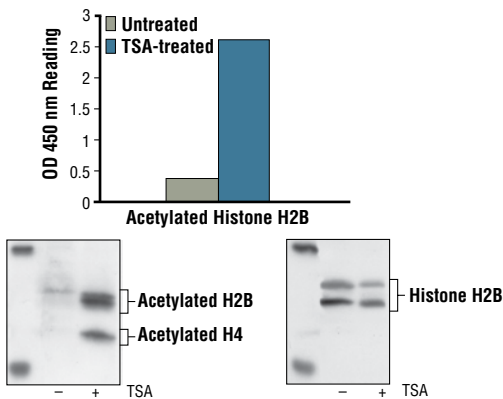


Figure 1: Treatment of COS cells with TSA causes accumulation of acetylation on Histone H2B, detected by Sandwich ELISA Kit #7178, but does not affect the level of total histone H2B protein, detected by Western analysis. OD 450 readings are shown in the top figure, while the corresponding Western blots using the Acetylated Lysine Mouse mAb (Ac-K-103) #9681 (left panel) or Histone H2B Antibody #2722 (right panel), are shown in the bottom figure.



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:

- (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.

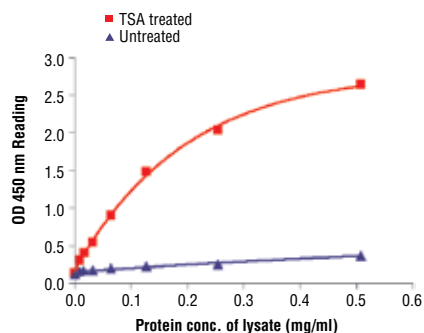


Figure 2: The relationship between protein concentration of lysates from untreated and TSA treated COS cells and kit assay optical density readings. COS cells (80% confluence) were treated with TSA (0.4 μ M overnight) and then lysed.

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_3VO_4 , 1 $\mu\text{g}/\text{ml}$ 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 cm²) 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. (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.

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. 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 C4.
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 C4.
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.