Store at 4°C

#7218

PathScan® Acetyl-Histone H2B (Lys5) Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 08/29/13

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

Species Cross-Reactivity: H, M, Mk

Introduction: The PathScan® Acetyl-Histone H2B (Lys5) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of histone H2B when acetylated at Lys5. A Histone H2B Antibody* has been coated onto the microwells. After incubation with cell lysates, histone H2B protein (acetylated and non-acetylated) is captured by the coated antibody. Following extensive washing, Acetyl-Histone H2B (Lys5) Antibody* is added to detect acetylated Lys5 on the histone H2B protein. Anti-rabbit IgG HRP-linked antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of histone H2B acetylated at Lys5.

* Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Acetyl-Histone H2B (Lys5) Sandwich ELISA Kit detects endogenous levels of histone H2B when acetylated at Lys5. As shown in Figure 1 using the Acetyl-Histone H2B (Lys5) Sandwich ELISA Kit #7218, a high level of acetylation at Lys5 on histone H2B is detected in NIH/3T3 cells when treated with trichostatin A (TSA). The level of total histone H2B (acetylated and non-acetylated) remains unchanged as shown by Western analysis (Figure 1). Similar results are obtained when COS and Jurkat cells are treated with TSA (data not shown).

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 Lys9, 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).

Products Included	Volume	Solution Color
Histone H2B Antibody Coated Microwells*	96 tests	
Acetyl-Histone H2B (Lys5) Detection Antibody	11 ml	green
Anti-rabbit IgG HRP-Linked Antibody	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).

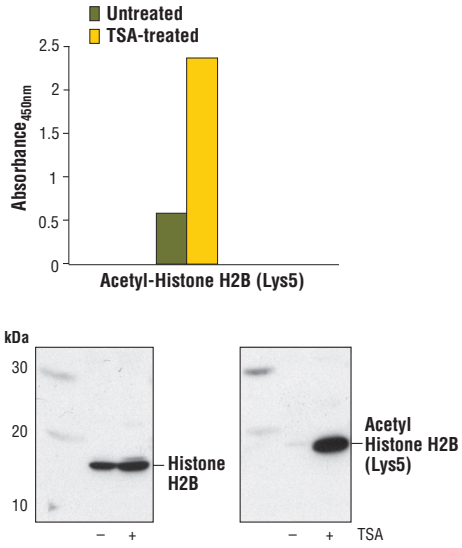

Figure 1. Treatment of NIH/3T3 cells with trichostatin A (TSA) increases the acetylation of Histone H2B at Lys5 detected by PathScan® Acetyl-Histone H2B (Lys5) Sandwich ELISA Kit #7218. TSA treatment does not affect the level of histone H2B that is detected by Western analysis. NIH/3T3 cells (70-80% confluent) were treated for 16-18 hours with 0.4 µM TSA at 37°C. Absorbance readings at 450 nm are shown in the top figure while the corresponding Western blots using Histone H2B Antibody #2722 (left panel) or Acetyl-Histone H2B (Lys5) Antibody #2574 (right panel) are shown in the bottom figure.

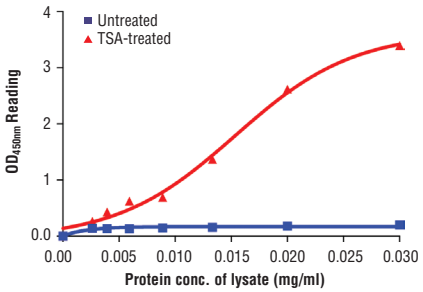

Figure 2. The relationship between the protein concentration of lysates from untreated and TSA-treated HeLa cells and kit assay optical density readings. HeLa cells were treated with TSA (4uM overnight). An acid extraction was performed for cell lysis in the presence of 5mM sodium butyrate.

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

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.

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.

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, 1µg/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 phenylmethylsulfonyl 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.