Store at 4°C

#7864

PathScan® Pan-Methyl-Histone H3 (Lys9) Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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New 06/08

Species Cross-Reactivity: H, M, Mk

Introduction: The PathScan® Pan-Methyl-Histone H3 (Lys9) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of histone H3 when methylated at Lys9. A Pan-Methyl-Histone H3 (Lys9) Rabbit Antibody* has been coated onto the microwells. After incubation with cell lysates, methylated-histone H3 (Lys9) is captured by the coated antibody. Following extensive washing, biotinylated Histone H3 Rabbit Antibody* is added to detect the histone H3 protein. HRP-linked streptavidin 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 H3 methylated at Lys9.

* Antibodies in kit are custom formulations specific to kit.

Companion Products:

Histone H3 Antibody #9715

Pan-Methyl-Histone H3 (Lys9) Antibody #4069

PathScan® Total Histone H3 Sandwich ELISA Kit #7253

PathScan® Acetyl-Histone H3 (Lys9) Sandwich ELISA Kit #7121

PathScan® Acetyl-Histone H3 (Lys18) Sandwich ELISA Kit #7122

PathScan® Mono-Methyl-Histone H3 (Lys4) Sandwich ELISA Kit #7123

PathScan® Di-Methyl-Histone H3 (Lys4) Sandwich ELISA Kit #7124

PathScan® Tri-Methyl-Histone H3 (Lys4) Sandwich ELISA Kit #7125

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

Specificity/Sensitivity: CST's PathScan® Pan-Methyl-Histone H3 (Lys9) Sandwich ELISA Kit #7864 detects endogenous levels of histone H3 when methylated at Lys9. As shown in Figure 1 using the Pan-Methyl-Histone H3 (Lys9) Sandwich ELISA Kit #7864, a high level of methylation at Lys9 is detected on Histone H3 in NIH/3T3 cells. These levels are unchanged in response to TSA-treatment. The level of total histone H3 (modified and unmodified) 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).

Note: For this assay, it is recommended that lysates be thoroughly sonicated to ensure complete extraction of Histone H3 and an accurate absorbance reading.

Products Included	Volume	Solution Color
Pan-Methyl-Histone H3 (Lys9) Rabbit Antibody Coated Microwells*	96 tests	
Histone H3 Rabbit Detection Antibody	11 ml	green
HRP-Linked Streptavidin	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).

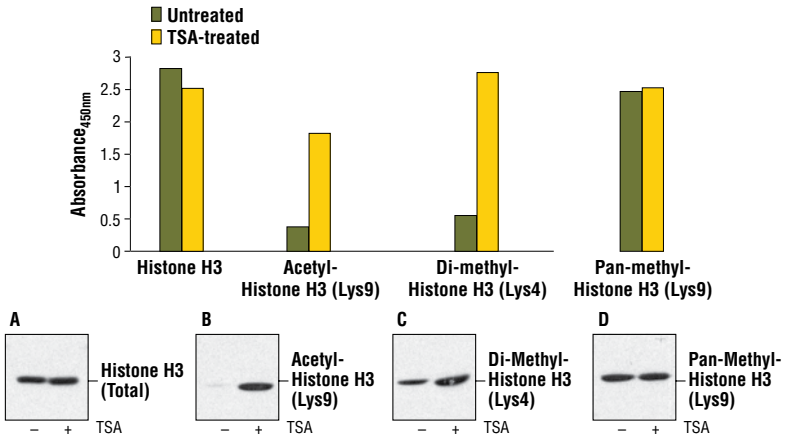

Figure 1. Treatment of NIH/3T3 cells with trichostatin A (TSA) increases the acetylation of Histone H3 at Lys 9, detected by PathScan® Acetyl-Histone H3 (Lys9) Sandwich ELISA Kit #7121, and the di-methylation of Histone H3 at Lys4, detected by PathScan® Di-Methyl-Histone H3 (Lys4) Sandwich ELISA Kit #7124. However, TSA treatment does not affect the level of general methylation at Lys9, detected by PathScan® Pan-Methyl-Histone H3 (Lys9) Sandwich ELISA Kit #7864, or the level of total Histone H3, detected by Pathscan® Total Histone H3 Sandwich ELISA Kit #7253. 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 H3 Antibody #9715 (panel A), Acetyl-Histone H3 (Lys9) Antibody #9671 (panel B), Di-Methyl-Histone H3 (Lys4) (C64G9) Rabbit mAb #9725 (panel C) or Pan-Methyl-Histone H3 (Lys9) Antibody #4069 (panel D) 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 Lys5, 12, 15 and 20 (4,7). Histone H3 is primarily acetylated at Lys9, 14, 18 and 23. 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).

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.

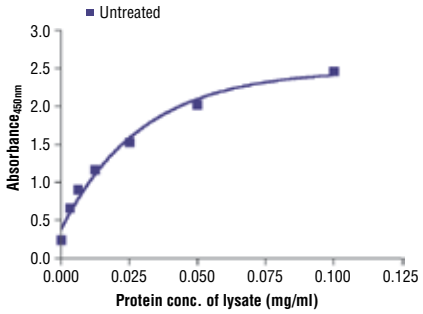

Figure 2. The relationship between the protein concentration of the lysate from untreated NIH/3T3 cells and the absorbance at 450 nm is shown.

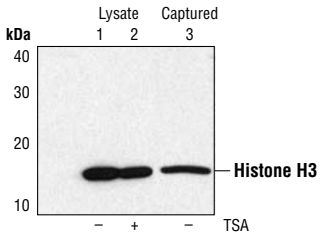

Figure 3. Kit specificity as demonstrated by Western analysis of the ELISA microwell captured protein. Lysates were prepared from NIH/3T3 cells and incubated in microwells coated with the Pan-Methyl-Histone H3 (Lys9) capture antibody. Wells were washed, and the captured protein was solubilized in SDS gel loading buffer. Western analysis of NIH/3T3 cell starting lysate (lanes 1 & 2) and the captured protein (lane 3) was performed using Histone H3 Antibody #9715. The major band detected in the captured material corresponds to Histone H3 methylated at Lys9 (lane 3).

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.