

#7868C Store at 4°C

PathScan® Di-Methyl Histone H3 (Lys36) Sandwich ELISA Kit



1 Kit
 (96 assays)

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

Swiss-Prot Acc. #P68431

Species Cross-Reactivity: H, Mk

Description: The PathScan® Di-Methyl-Histone H3 (Lys36) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of histone H3 when di-methylated at Lys36. A Di-Methyl-Histone H3 (Lys36) Rabbit Antibody* has been coated onto the microwells. After incubation with cell lysates, Di-methyl-histone H3 (Lys36) is captured by the coated antibody. Following extensive washing, Histone H3 Mouse Antibody* is added to detect the histone H3 protein. Anti-Mouse IgG, HRP-linked Antibody is then used to recognize the bound detection antibody. 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 di-methylated at Lys36.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Di-Methyl-Histone H3 (Lys36) Sandwich ELISA Kit #7868 detects endogenous levels of histone H3 when di-methylated at Lys36. As shown in Figure 1 using the Di-Methyl-Histone H3 (Lys36) Sandwich ELISA Kit #7868, a high level of di-methylation at Lys36 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. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background: Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of DNA wound around eight core histone proteins (two each of 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, 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, 23, 27, and 56. Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in

Products Included	Volume	Solution Color
Di-Methyl-Histone H3 (Lys36) Rabbit mAb Coated Microwells*	96 tests	
Histone H3 Mouse Detection Antibody	1 each	Green (Lyophilized)
Anti-mouse IgG, HRP-linked Antibody	1 each	Red (Lyophilized)
Detection Antibody Diluent	11 ml	Green
HRP Diluent	11 ml	Red
TMB Substrate #7004	11 ml	Colorless
STOP Solution #7002	11 ml	Colorless
Sealing Tape	2 sheets	
20X ELISA Wash Buffer	25 ml	Colorless
ELISA 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).

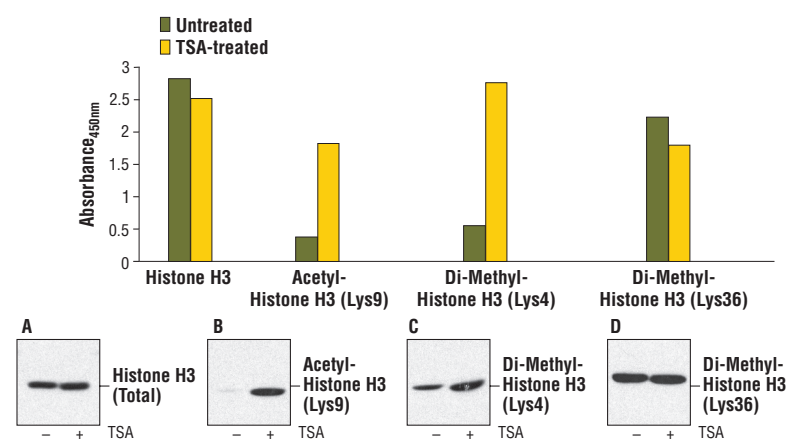


Figure 1. Treatment of NIH/3T3 cells with trichostatin A (TSA) increases the acetylation of Histone H3 at Lys 9, detected by PathScan® Acetyl-Histone H3 (Lys9) Sandwich ELISA Kit #7121, and the di-methylation of Histone H3 at Lys4, detected by PathScan® Di-Methyl-Histone H3 (Lys4) Sandwich ELISA Kit #7124. However, TSA treatment does not affect the level of di-methylation at Lys36, detected by PathScan® Di-Methyl-Histone H3 (Lys36) Sandwich ELISA Kit #7868, 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 Di-Methyl-Histone H3 (Lys36) Antibody #9758 (panel D) are shown in the bottom figure.

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 at 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 at Thr3 of H3 in

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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prophase and its dephosphorylation during anaphase (11).

Background References:

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- (2) Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637-41.
- (3) Strahl, B.D. and Allis, C.D. (2000) *Nature* 403, 41-5.
- (4) Cheung, P. et al. (2000) *Cell* 103, 263-71.
- (5) Bernstein, B.E. and Schreiber, S.L. (2002) *Chem Biol* 9, 1167-73.
- (6) Jaskelioff, M. and Peterson, C.L. (2003) *Nat Cell Biol* 5, 395-9.
- (7) Thorne, A.W. et al. (1990) *Eur J Biochem* 193, 701-13.
- (8) Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348-60.
- (9) Goto, H. et al. (1999) *J Biol Chem* 274, 25543-9.
- (10) Preuss, U. et al. (2003) *Nucleic Acids Res* 31, 878-85.
- (11) Dai, J. et al. (2005) *Genes Dev* 19, 472-88.

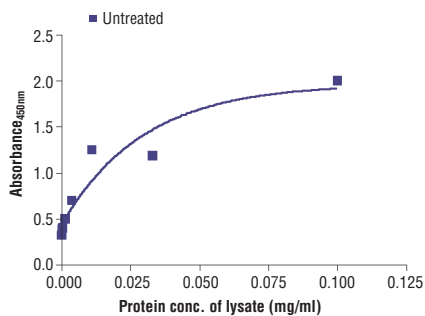


Figure 2. The relationship between the protein concentration of the lysate from untreated NIH/3T3 cells and the absorbance at 450 nm is shown.

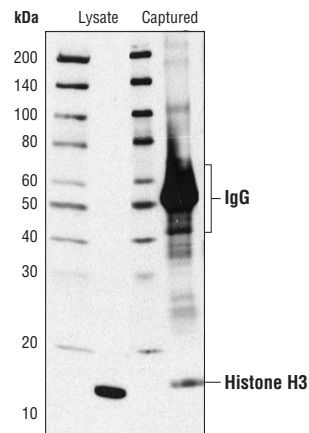


Figure 3. Kit specificity as demonstrated by Western analysis of the ELISA microwell captured protein. Lysates were prepared from NIH/3T3 cells and incubated in microwells containing immobilized Di-Methyl-Histone H3 (Lys36) capture antibody. Wells were washed, and the captured protein was solubilized in SDS gel loading buffer. Western analysis of NIH/3T3 cell starting lysate and the captured protein was performed using Histone H3 Antibody #9715. The major band detected in the captured material corresponds to Histone H3 di-methylated at Lys36.

PathScan® Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

- 1. Microwell strips:** Bring all to room temperature before use.
- 2. Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- 3. HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- 4. Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- 5. HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- 6. Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 7. 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- 8. Cell Lysis Buffer:** Cell Lysis Buffer: 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) immediately before use.
- 9. TMB Substrate (#7004).**
- 10. STOP Solution (#7002).**

**Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.*

B Preparing Cell Lysates

For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 rpm) 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.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) 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. Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
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 reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min 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 min after adding STOP Solution.
 - b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.