

PathScan® Phospho-Histone H3 (Ser10) Sandwich ELISA Kit



Cell Signaling
TECHNOLOGY®

✓ 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. 01/17/08

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

Species Cross-Reactivity: H, M

Introduction: CST's PathScan® Phospho-Histone H3 (Ser10) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Phospho-Histone H3 (Ser10) protein. A Histone H3 Antibody (#9715*) has been coated onto the microwells. After incubation with cell lysates, both non-phospho- and phospho-Histone H3 proteins are captured by the coated antibody. Following extensive washing, a Biotinylated Phospho-Histone H3 (Ser10) Antibody (#9701*) is added to detect the captured phospho-Histone H3 (Ser10) protein. HRP-linked Streptavidin 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 Phospho-Histone H3 (Ser10) protein.

* Antibodies in this kit are custom formulations specific to the kit.

Companion Products:

Histone H3 Antibody #9715

Phospho-Histone H3 (Ser10) Antibody #9701

Calyculin A (Serine/Threonine Phosphatase Inhibitor) #9902

Specificity/Sensitivity: CST's PathScan® Phospho-Histone H3 (Ser10) Sandwich ELISA Kit detects endogenous levels of Phospho-Histone H3 (Ser10). Using this Sandwich ELISA Kit #7155, Phospho-Histone H3 (Ser10) is detected when treated with Calyculin A in NIH 3T3 cells. However, the levels of Histone H3 remains unchanged, as shown by Western analysis using the Histone H3 Antibody #9715 (Figure 1). 293 cells treated with Calyculin A show similar results (data not shown).

Products Included	Volume	Solution Color
Histone H3 Antibody Coated Microwells*	96 tests	
Biotinylated Phospho-Histone H3 (Ser10) 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).

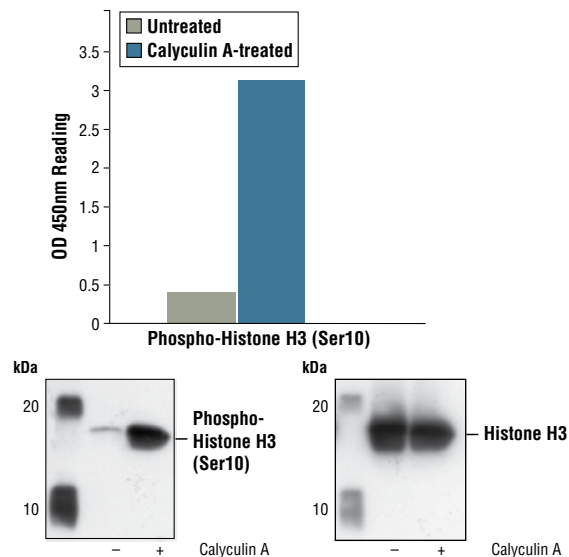


Figure 1: Treatment of NIH/3T3 cells with Calyculin A causes accumulation of phospho-histone H3 (Ser10), detected by Sandwich ELISA kit #7155, but does not affect the level of total histone H3 protein, detected by Western analysis. OD 450 readings are shown in the top figure, while the corresponding Western blot using Phospho-Histone H3 (Ser10) Antibody #9701 or Histone H3 Antibody #9715, is 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 (2,3). 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.

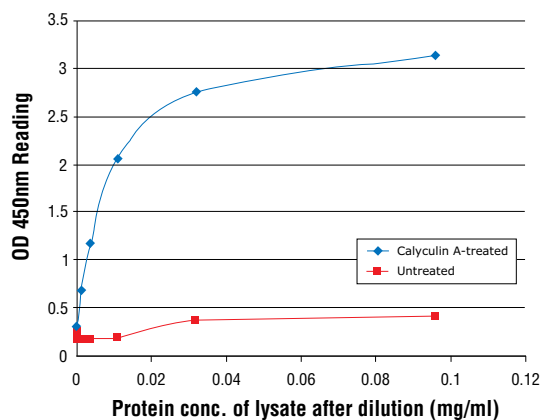


Figure 2: Linear relationship between protein concentration of lysates from untreated and Calyculin A-treated NIH/3T3 cells and kit assay optical density readings. NIH/3T3 cells (80% confluence) were serum-starved overnight, and serum was added back for 15 minutes followed by treatment with Calyculin A (0.1 μ M for 15 minutes).

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 Na₃VO₄, 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.