Store at 4°C

#7303

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

☑ 1 Kit
(96 assays)
Low volume microplate

Cell Signaling TECHNOLOGY®

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New 12/11

Entrez-Gene ID # 8350
Swiss-Prot Acc. #P68431

Species Cross-Reactivity: H, M

Description: The PathScan® Phospho-Histone H3 (Ser10) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-histone H3 (Ser10) protein with a chemiluminescent readout. Chemiluminescent ELISAs often have a wider dynamic range and higher sensitivity than conventional chromogenic detection. This chemiluminescent ELISA, which is offered in low volume microplates, shows increased signal and sensitivity while using smaller samples. A Histone H3 Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, both nonphospho- and phospho-histone H3 proteins are captured by the coated antibody. Following extensive washing, Biotinylated Phospho-Histone H3 (Ser10) Detection mAb 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. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of phospho-histone H3 (Ser10) protein.

Antibodies in kit are custom formulations specific to kit.

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 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 prophase and its dephosphorylation during anaphase (11).

Specificity/Sensitivity: The PathScan® Phospho-Histone H3 (Ser10) Chemiluminescent Sandwich ELISA Kit detects endogenous levels of phospho-histone H3 (Ser10). This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Products Included	Volume	Solution Color
Histone H3 Rabbit mAb Coated Microwells*	96 tests	
Biotinylated Phospho-Histone H3 (Ser10) Detection mAb	5.5 ml	Green
HRP-Linked Streptavidin	5.5 ml	Red
Luminol/Enhancer Solution	3 ml	Colorless
Stable Peroxide Buffer	3 ml	Colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	Colorless
Sample Diluent	25 ml	Blue
**Cell Lysis Buffer #9803	15 ml	Yellowish

Low volume microplate * 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).

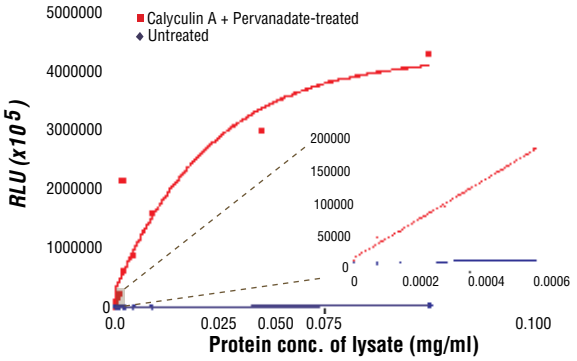

Figure 1. The relationship between protein concentration of lysates from Jurkat cells, untreated or treated with calyculin A (100 nM) and pervanadate (1 mM), and immediate light generation with chemiluminescent substrate is shown. Cells were lysed using Cell Lysis Buffer (10X) #9803. Graph inset corresponding to the shaded area shows high sensitivity and a linear response at the low protein concentration range.

Background References:

(1) Workman, J.L. and Kingston, R.E. (1998) *Annu Rev Biochem* 67, 545-79.
(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.

Chemiluminescent ELISA Protocol

NOTE: This chemiluminescent ELISA is offered in low volume microplate. Samples and reagents only require 50 µl per microwell.

A Required Reagents

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: (10X Cell Lysis Buffer #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). **Note:** CST recommends adding 1 mM PMSF immediately before use.

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 50 µl of Sample Diluent (supplied in each Pathscan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 50 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 50 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 room temperature (RT). 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, 150 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 dry completely at any time.
   - **d.** Clean the underside of all wells with a lint-free tissue.
5. Add 50 µl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at room temperature.
6. Repeat wash procedure as in Step C4.
7. Add 50 µl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at room temperature.
8. Repeat wash procedure as in Step C4.
9. Prepare Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50 µl of the Working Solution to each well.

Use a plate-based luminometer to measure Relative Light Units (RLU) at 425nM within 1-10 minutes following addition of the substrate.

Optimal signal intensity is achieved when read within 10 minutes.