Revision 1

Store at +4C

#7303

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

Cell Signaling TECHNOLOGY®

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Species Cross Reactivity: H M

UniProt ID: #P68431

Entrez-Gene Id: #8350

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Quantity	Color	Storage Temp
Luminol/Enhancer Solution	84850	3 ml	Colorless	RT
Stable Peroxide Buffer	42552	3 ml	Colorless	RT
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-20C

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.

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.

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 posttranslational 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).

Background References

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ELISA Chemiluminescent

NOTE: Refer to product-specific datasheets or product webpage for assay incubation temperature. This /product/productDetail.jsp?productId=luminescent ELISA is offered in low volume microplate. Samples and reagents only require 50 µl per microwell.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS)**: (#9808) To prepare 1 L 1X PBS: add 50 ml 10X PBS to 950 ml dH_2O, mix.
2. Bring all microwell strips to room temperature before use.
3. Prepare 1X wash buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in dH_2O.
4. **1X Cell Lysis Buffer**: 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH_2O, mix. Buffer can be stored at 4°C for short-term use (1–2 weeks).

   Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

5. **20X LumiGLO® Reagent and 20X Peroxide**: (#7003).

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 (~1,200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth medium 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 in the storage bag and stored at 4°C immediately.
2. Cell lysates can be used undiluted or diluted with sample diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets or product webpage for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
3. Add 50 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 room temperature. Alternatively, the plate can be incubated overnight at 4°C.
4. **Gently remove the tape and wash wells**:
    1. Discard plate contents into a receptacle.
    2. Wash 4 times with 1X Wash Buffer, 150 µl each time per well.
    3. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to dry completely at any time.

    4. 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 at room temperature for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 50 µl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate at room temperature for 30 min.
8. Repeat wash procedure (Section C, Step 4).
9. Prepare detection reagent working solution by mixing equal parts 2X LumiGLO® Reagent and 2X Peroxide.
10. Add 50 µl of the detection reagent working solution to each well.
11. Use a plate-based luminometer set at 425 nm to measure Relative Light Units (RLU) within 1–10 min following addition of the substrate.
    1. Optimal signal intensity is achieved when read within 10 min.

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