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#7178

## PathScan® Acetyl-Histone H2B Sandwich ELISA Kit

**Species Cross Reactivity:** H M Mk  
**UniProt ID:** #P33778  
**Entrez-Gene Id:** #3018

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

| Product Includes  | Product # | Quantity | Color               | Storage Temp |
|---|-----------|----------|---------------------|--------------|
| Acetylated-Lysine Rabbit Detection mAb                  | 13968     | 1 ea     | Green (Lyophilized) | +4C          |
| Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated) | 13272     | 1 ea     | Red (Lyophilized)   | +4C          |
| Detection Antibody Diluent                              | 13339     | 11 ml    | Green               | +4C          |
| HRP Diluent   | 13515     | 11 ml    | Red                 | +4C          |
| TMB Substrate   | 7004      | 11 ml    | Colorless           | +4C          |
| STOP Solution   | 7002      | 11 ml    | Colorless           | +4C          |
| 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         |

Kit contents scale proportionally with size, except sealing tape.

Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

### Description

The PathScan® Acetyl-Histone H2B Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of acetylated lysines on Histone H2B. A Histone H2B Antibody has been coated onto the microwells. After incubation with cell lysates, Histone H2B is captured by the coated antibody. Following extensive washing, an Acetylated-Lysine Rabbit mAb is added to detect the acetylated lysines on the Histone H2B protein. Anti-rabbit IgG, HRP linked Antibody 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 acetylated Histone H2B.

Antibodies in kit are custom formulations specific to kit.

### Specificity/Sensitivity

CST's PathScan® Acetyl-Histone H2B Sandwich ELISA Kit detects endogenous levels of acetylated Histone H2B. Using this Sandwich ELISA Kit #7178, acetylated lysines on Histone H2B are detected when treated with TSA in Cos cells. However, the levels of total Histone H2B protein remain unchanged, as shown by Western blot analysis, using the Histone H2B Antibody #2722 (figure 1). Jurkat cells treated with TSA show similar results (data not shown). 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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#7178

# PathScan<sup>®</sup> Acetyl-Histone H2B Sandwich ELISA Kit



## ELISA Colorimetric

**NOTE:** Refer to product-specific datasheets or product webpage for assay incubation temperature.

### 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 PBS: add 50 ml 10X PBS to 950 ml dH<sub>2</sub>O, 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<sup>®</sup> Sandwich ELISA Kit) in dH<sub>2</sub>O.
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<sub>2</sub>O, 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.

**NOTE:** Refer to product-specific datasheet or webpage for lysis buffer recommendation.

5. **TMB Substrate:** (#7004).
6. **STOP Solution:** (#7002).

### 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<sup>6</sup> 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 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 in the storage bag and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with sample diluent (supplied in each PathScan<sup>®</sup> 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 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:**
  1. Discard plate contents into a receptacle.
  2. Wash 4 times with 1X wash buffer, 200 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 completely dry at any time.
  4. 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 at 37°C for 1 hr.

6. Repeat wash procedure (Section C, Step 4).
7. Add 100  $\mu$ l of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100  $\mu$ l 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  $\mu$ l 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**

1. **Visual Determination:** Read within 30 min after adding STOP solution.
2. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

posted June 2005

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