

# PathScan® Phospho-Aurora A (Thr288) Sandwich ELISA Kit

✓ 1 Kit  
(96 assays)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

Entrez-Gene ID #6790  
Swiss-Prot Acc. #O14965

## Species Cross-Reactivity: H

**Introduction:** The PathScan® Phospho-Aurora A (Thr288) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Aurora A when Thr288 is phosphorylated. An Aurora A Rabbit Antibody\* has been coated onto the microwells. After incubation with cell lysates, Aurora A (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Biotinylated Phospho-Aurora A (Thr288) Rabbit Detection Antibody\* is added to detect threonine phosphorylation of the captured Aurora A protein. 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 Aurora A phosphorylated on Thr288.

\* Antibodies in kit are custom formulations specific to kit.

## Companion Products:

Aurora A/AIK (1G4) Rabbit mAb #4718

Aurora A/AIK Antibody #3092

Phospho-Aurora A (Thr288)/Aurora B (Thr232)/Aurora C (Thr198) (D13A11) Rabbit mAb #2914

Cell Lysis Buffer (10X) #9803

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

BSA #9998

TMB Substrate #7004

STOP Solution #7002

**Specificity/Sensitivity:** CST's PathScan® Phospho-Aurora A (Thr288) Sandwich ELISA Kit #7114 detects Aurora A when Thr288 is phosphorylated during G2/M phase of the cell cycle. As shown in Figure 1, a significant induction of Phospho-Aurora A (Thr288) can be detected in HeLa cells treated with paclitaxel using the Phospho-Aurora A (Thr288) Sandwich ELISA Kit #7114. These high levels are abolished when paclitaxel-treated HeLa cells were lysed without addition of phosphatase inhibitors\* to the lysis buffer. The levels of total Aurora A protein (either phospho or nonphospho) detected by PathScan® Total Aurora A Sandwich ELISA Kit #7116 remain unchanged.

\* Phosphatase inhibitors include sodium pyrophosphate, β-glycerophosphate and Na<sub>3</sub>VO<sub>4</sub>.

Products Included	Volume	Solution Color
Aurora A Rabbit Antibody Coated Microwells*	96 tests	
Biotinylated Phospho-Aurora A (Thr288) Rabbit 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).

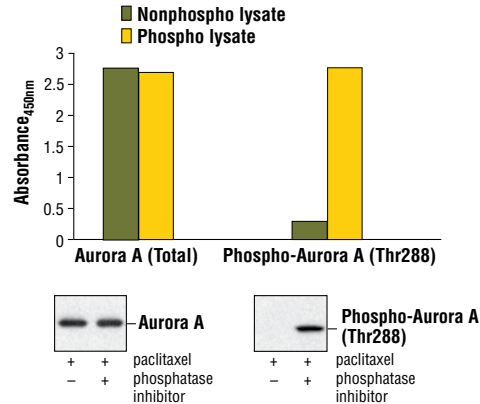


Figure 1. Induced phosphorylation of Aurora A in paclitaxel-treated HeLa cells lysed in the presence of phosphatase inhibitors (phospho lysate) is detected by PathScan® Phospho-Aurora A (Thr288) Sandwich ELISA Kit #7114 (upper, right). In contrast, a low level of phospho-Aurora A protein is detected in paclitaxel-treated HeLa cells lysed without addition of phosphatase inhibitors to the lysis buffer (nonphospho lysate). Similar levels of Aurora A protein from either nonphospho or phospho lysates are detected by PathScan® Total Aurora A Sandwich ELISA Kit #7116 (upper, left). The absorbance readings at 450 nm are shown in the top figure, while the corresponding Western blots using Aurora A Antibody #3092 (left panel) or Phospho-Aurora A (Thr288) Rabbit mAb #3079 (right panel) are shown in the bottom figure.



**Background:** Aurora kinases belong to a highly conserved family of mitotic serine/threonine kinases with three members identified among mammals: Aurora A, Aurora B and Aurora C (1,2). Studies on the temporal expression pattern and subcellular localization of Aurora kinases in mitotic cells suggest an association with mitotic structure. Their functional influences span from G2 to cytokinesis and may be involved in key cell cycle events such as centrosome duplication, chromosome bi-orientation and segregation, cleavage furrow positioning and ingression (3). Aurora A is detected at the centrosomes, along mitotic spindle microtubules and in the cytoplasm of mitotically proliferating cells. Aurora A protein levels are low during G1 and S phases and peak during the G2/M phase of the cell cycle. Phosphorylation of Aurora A at Thr288 in its catalytic domain increases kinase activity. Aurora A is involved in centrosome separation, maturation and spindle assembly and stability. Expression of Aurora B protein also peaks during the G2/M phase of the cell cycle, while kinase activity peaks at the transition from metaphase to the end of mitosis. Aurora B associates with chromosomes during prophase prior to relocalizing to the spindle at anaphase. Aurora B regulates chromosome segregation through the control of microtubule-kinetochore attachment and cytokinesis. Expression of both Aurora A and Aurora B during the G2/M phase transition is tightly coordinated with histone H3 phosphorylation (4,5), while overexpression of both kinases is seen in a variety of human cancers (2,4). Aurora C localizes to the centrosome from anaphase to cytokinesis and both mRNA and protein levels peak during G2/M phase. Although typical Aurora C expression is limited to the testis, overexpression of Aurora C is detected in various cancer cell lines (6).

#### Background References:

- (1) Warner, S.L. et al. (2003) *Mol. Cancer Ther.* 2, 589–595.
- (2) Katayama, H. et al. (2003) *Cancer Metastasis Rev.* 22, 451–464.
- (3) Andrews, P.D. et al. (2003) *Curr. Opin. Cell Biol.* 15, 672–683.
- (4) Pascreau, G. et al. (2003) *Prog. Cell Cycle Res.* 5, 369–374.
- (5) Crosio, C. et al. (2002) *Mol. Cell. Biol.* 22, 874–885.
- (6) Kimura, M. et al. (1999) *J. Biol. Chem.* 274, 7334–7340.

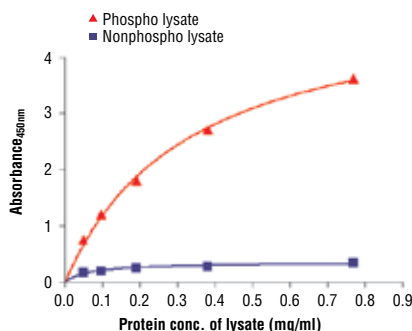


Figure 2: The relationship between protein concentration of phospho or nonphospho Aurora A lysates and the absorbance at 450 nm is shown. Unstarved HeLa cells (85% confluence) treated with paclitaxel (100 nM) for 20 hours were harvested and then lysed in the absence or presence of phosphatase inhibitor.

## 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 Na<sub>3</sub>VO<sub>4</sub>, 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.