Store at 4°C and –20°C

#7117

# PathScan® Total Aurora A Sandwich ELISA Antibody Pair

☑ 1 Kit
(4 X 96 assays)

Cell Signaling TECHNOLOGY®

**Orders** ■ 877-616-CELL (2355)
orders@cellsignal.com
**Support** ■ 877-678-TECH (8324)
info@cellsignal.com
**Web** ■ www.cellsignal.com

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

**Species Cross-Reactivity: H**

**Description:** CST's PathScan® Total Aurora A Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Total Aurora A Sandwich ELISA Kit #7116. Capture and Detection Antibodies (100X stocks) and HRP-conjugated Secondary Antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The Aurora A Rabbit Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added followed by Aurora A Mouse Detection Antibody and HRP-conjugated Secondary Antibody. HRP substrate (TMB) is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of Aurora A protein.

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

**Reagents not supplied:**
Phosphate Buffered Saline (PBS-20X) #9808

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

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA

96 Well Microplates**

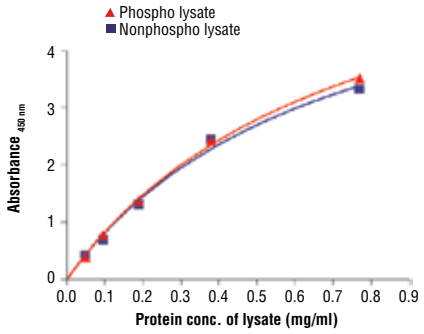
Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

**Notes:** Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

**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.

| Products Included | Volume | Cap Color | Storage |
|---|---|---|---|
| Aurora A Rabbit Capture Antibody (100X) | 0.4 ml | Pink | 4°C |
| Aurora A Mouse Detection Antibody (100X) | 0.4 ml | Blue | 4°C |
| Anti-mouse IgG, HRP-Linked Antibody (1000X) | 0.04 ml | Yellow | –20°C |




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

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).

**Storage:** Capture and Detection Antibodies are stored at 4°C. HRP-conjugated secondary antibody is stored at -20°C.

**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

PathScan® Phospho-Aurora A (Thr288) Sandwich ELISA Kit #7114

PathScan® Total Aurora A Sandwich ELISA Kit #7116

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

Anti-mouse IgG, HRP-linked Antibody #7076

**For application specific protocols please see the web page for this product at www.cellsignal.com.**

**Please visit www.cellsignal.com for a complete listing of recommended companion products.**

**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.



**Applications Key:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

**Species Cross-Reactivity Key:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.