Revision 1

Store at +4C

#7116

PathScan® Total Aurora A Sandwich ELISA Kit

Cell Signaling TECHNOLOGY®

Orders: 877-616-CELL (2355) orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

UniProt ID: #O14965 **Entrez-Gene Id:** #6790

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

Product Includes	Product #	Quantity	Color	Storage Temp
TMB Substrate	7004	11 ml	Colorless	+4C
STOP Solution	7002	11 ml	Colorless	+4C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Sealing Tape	54503	2 ea		+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® Total Aurora A Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total Aurora A protein. 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, an Aurora A Mouse Detection Antibody is added to detect the captured phospho and nonphospho Aurora A protein. Anti-mouse IgG, HRP-linked Antibody 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 total Aurora A protein.
Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity

CST's PathScan® Total Aurora A Sandwich ELISA Kit #7116 detects endogenous levels of total Aurora A proteins. 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 during the G2/M phase of the cell cycle. Western analysis of protein captured in microwells coated with Aurora A antibody shows a single band corresponding to Aurora A protein (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.
* Phosphatase inhibitors include sodium pyrophosphate, β-glycerophosphate and Na_3VO_4.

Background

Aurora kinases belong to a highly conserved family of mitotic serine/threonine kinases with three members identified among mammals: Aurora A, B, and C (1,2). Studies on the temporal expression pattern and subcellular localization of Aurora kinases in mitotic cells suggest an association with mitotic structure. Aurora kinase functional influences span from G2 phase 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; Aurora B 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); research investigators have observed overexpression of these kinases 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, research studies report 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, 589-95.
2. Katayama, H. et al. (2003) *Cancer Metastasis Rev* 22, 451-64.
3. Andrews, P.D. et al. (2003) *Curr Opin Cell Biol* 15, 672-83.
4. Pascreau, G. et al. (2003) *Prog Cell Cycle Res* 5, 369-74.
5. Crosio, C. et al. (2002) *Mol Cell Biol* 22, 874-85.
6. Kimura, M. et al. (1999) *J Biol Chem* 274, 7334-40.

Trademarks and Patents

Limited Uses

Revision 1

#7116

PathScan® Total Aurora A 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_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.

   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 10^6 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 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® 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 µl 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 µl 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 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**
    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

revised November 2013