Revision 1

Store at 4C and -20C

#7117

PathScan® Total Aurora A Sandwich ELISA Antibody Pair

Cell Signaling TECHNOLOGY®

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Species Cross Reactivity:	UniProt ID:	Entrez-Gene Id:
H	#O14965	#6790

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

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.

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.

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Limited Uses

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ELISA Antibody Pair

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 20X PBS to 950 ml dH_2O, mix.
2. **Wash Buffer**: 1X PBS/0.05% Tween® 20, (20X PBST #9809).
3. **Blocking Buffer**: 1X PBS/0.05% Tween® 20, 1% BSA.
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. **Bovine Serum Albumin (BSA)**: (#9998).
6. **TMB Substrate**: (#7004).
7. **STOP Solution**: (#7002)

   NOTE: Reagents should be made fresh daily.

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. Coating Procedure

1. Rinse microplate with 200 µl of dH_2O, discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in 1X PBS. For a single 96 well plate, add 100 µl of capture antibody stock to 9.9 ml 1X PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17–20 hr).
3. **After overnight coating, gently uncover plate and wash wells**:
    1. Discard plate contents into a receptacle.
    2. Wash four times with wash buffer, 200 µl each time per well. 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.
    3. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of blocking buffer/well, cover plate, and incubate at 37°C for 2 hr.
5. After blocking, wash plate (Section C, Step 3). Plate is ready to use.

D. Test Procedure

1. Lysates can be used undiluted or diluted in blocking buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hr.
2. Wash plate (Section C, Step 3).
3. Dilute detection antibody 1:100 in blocking buffer. For a single 96 well plate, add 100 µl of detection antibody Stock to 9.9 ml of blocking buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hr.
4. Wash plate (Section C, Step 3).
5. Secondary antibody, either streptavidin anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in blocking buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of blocking buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30 min.
6. Wash plate (Section C, Step 3).
7. Add 100 µl of TMB substrate per well. Cover and incubate at 37°C for 10 min.
8. Add 100 µl of STOP solution per well. Shake gently for a few seconds.
9. Read plate on a microplate reader at absorbance 450 nm.
    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 January 2008

revised Sepetember 2013