Store at -80°C

#7512

Aurora B Kinase

☑ 5 µg

Cell Signaling TECHNOLOGY®

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Description: Purified recombinant Aurora B kinase (Ala2-Ala344), supplied as a GST fusion protein.

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 pattern of expression and subcellular localization of Aurora kinases in mitotic cells suggest an association with mitotic structure. Their functional influences span from G2 through to cytokinesis and may be involved in key cell cycle events such as centrosome duplication, chromosome biorientation and segregation, cleavage furrow positioning and ingression (3). Aurora A is detected in mitotically proliferating cells at the centrosomes, along microtubules of the mitotic spindle and in cytoplasm. Its protein levels are low during G1 and S phases and peak during the G2/M phase of the cell cycle. Phosphorylation of Thr288 in its catalytic domain increases kinase activity. Aurora A is involved in centrosome separation, maturation and spindle assembly and stability. Overexpression of Aurora A has been detected in human breast, bladder, colon, ovarian and pancreatic cancers (2,4). The expression of Aurora B also peaks during the G2/M phase of the cell cycle and the kinase activity peaks at the transition from metaphase to the end of mitosis. Aurora B associates with chromosomes during prophase and then relocalizes to the spindle at anaphase. Aurora B regulates chromosome segregation through the control of microtubule-kinetochore attachment and cytokinesis. Aurora B overexpression is also detected in a variety of human cancers (2,4). The expression of both Aurora A and Aurora B is tightly coordinated with histone H3 phosphorylation during the G2/M phase transition (4,5). Aurora C localizes on the centrosome from anaphase to cytokinesis and expression of both mRNA and protein levels peaks during G2/M phase. Although the tissue distribution of Aurora C shows that its expression is limited to the testis, overexpression of Aurora C is detected in various cancer cell lines (6).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human Aurora B (Ala2-Ala344) (GenBank Accession No. NM_004217) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-Aurora B fusion protein is 70 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Silver stain and Western blot [Fig.1]. Aurora B kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure Aurora B activity using HTScan™ Aurora B Kinase Assay Kit #7513 [Fig.3].

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.

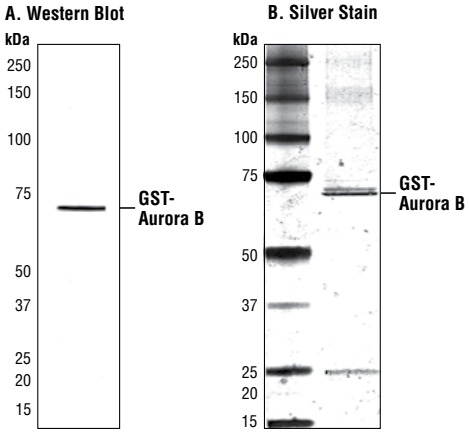

Figure 1. The purity of the GST-Aurora B fusion protein was analyzed using SDS/PAGE followed by anti-Aurora B Western blot (A) or Silver stain (B).

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

HTScan™ Aurora B Kinase Assay Kit #7513

PLK (Ser137) Biotinylated Peptide #1300

Phospho-PLK (Ser137) Antibody #5070

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

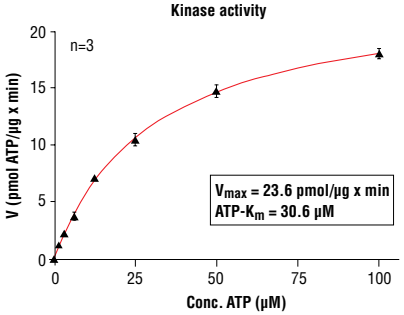

Figure 2. Aurora B kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: Tetra (LRRLSLG), 5 µg/50 µl and 200 ng/50 µl Recombinant Aurora B.

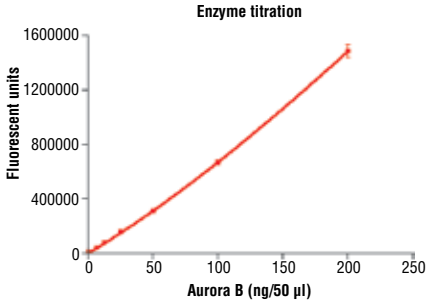

Figure 3. Dose dependence curve of Aurora B kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of substrate peptide (#1300) by Aurora B kinase. In a 50 µl reaction, increasing amounts of Aurora B and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for Aurora B Kinase Assay

***IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-PLK (Ser137) Antibody #5070
5. Kinase Buffer (10X) #9802
6. ATP (10 mM) #9804
7. PLK (Ser137) Biotinylated Peptide #1300
8. DELFIA® Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
9. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
10. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

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B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 to make 2.5 ml 4X reaction buffer.
5. Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail ([enzyme]=8.0 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM β-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
1.5 µM peptide
100 ng Aurora B Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
    Please note: This protocol was validated using a PLK (Ser137) Biotinylated Peptide and Phospho-PLK (Ser137) Antibody diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
13. Incubate at 37°C for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled secondary antibody 1:1000 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com