

# Aurora A Kinase

✓ 5 µg



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant full-length human Aurora A (Met1-Ser403) kinase, 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 relocates 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 full-length human Aurora A (Met1-Ser403) (GenBank Accession No. NM\_003600) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

**Quality Control:** The theoretical molecular weight of the Aurora A fusion protein is 72 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Aurora A kinase activity was determined using a radiometric assay [Fig.2].

#### 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.

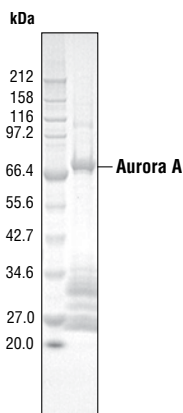


Figure 1. The purity of the Aurora A fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

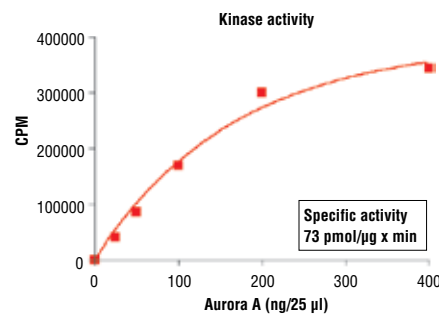


Figure 2. Aurora A kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl<sub>2</sub>, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 200 ng/µL, and recombinant Aurora A: variable.

## Protocol for Aurora A Kinase Assay

**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. Kinase Buffer (10X)

50 mM MOPS, pH 7.2  
25 mM  $\beta$ -glycerophosphate  
10 mM EGTA  
4 mM EDTA  
50 mM  $MgCl_2$   
0.5 mM DTT

#### 2. ATP (10 mM) #9804

#### 3. $^{32}P$ - $\gamma$ ATP

#### 4. MBP (0.5 $\mu$ g/ $\mu$ l)

### B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
2. Dilute [ $^{32}P$ ] ATP to 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP with 250  $\mu$ M ATP solution.
3. Transfer enzyme from  $-80^\circ C$  to ice. Allow enzyme to thaw on ice.
4. Dilute Aurora A protein to 20 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10  $\mu$ l diluted Aurora A kinase solution, 10  $\mu$ l MBP (0.5  $\mu$ g/ $\mu$ l), and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP solution.

### Final Assay Conditions

5 mM MOPS, pH 7.2  
2.5 mM  $\beta$ -glycerophosphate  
1 mM EGTA  
0.4 mM EDTA  
5 mM  $MgCl_2$   
0.05 mM DTT  
200 ng/ $\mu$ L MBP

6. After 15 minutes terminate reaction by spotting 20  $\mu$ l of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

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