

## Product: AURORA A, Active

Catalog #: 02-2068

Amount: 5 µg

### Product Description

Recombinant full-length mouse AURORA A was expressed in Sf9 cells using an N-terminal GST tag. The gene accession number is [NM\\_011497](#).

### Gene Aliases

AURKA, STK6; STK15; AIK; ARK1; AURA; BTAK; AURORA2

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

### Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

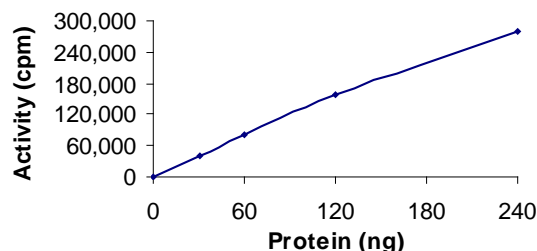
### Scientific Background

AURORA A belongs to a multigenic family of mitotic serine/threonine kinases which are involved in the control of chromosome segregation. AURORA A is involved in centrosome separation, duplication and maturation as well as in bipolar spindle assembly and stability (1). AURORA A is expressed and active at the highest level during G2-M phase of the cell cycle. Overexpression of AURORA A has been found to be correlated with the grade of various human solid tumours. Ectopic AURORA A overexpression in any culture cell line leads to polyploidy and centrosome amplification (2).

### References

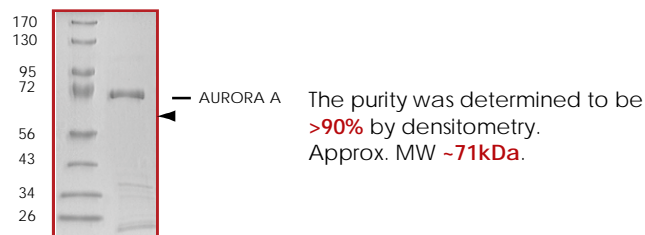
1. Dutertre, S. et al: On the role of aurora-A in centrosome function. *Oncogene*. 2002 Sep 9;21(40):6175-83.
2. Katayama, H. et al: The Aurora kinases: role in cell transformation and tumorigenesis. *Cancer Metastasis Rev*. 2003 Dec;22(4):451-64.

### Specific Activity



The specific activity of AURORA A was determined to be **69 nmol /min/mg** as per activity assay protocol.

### Purity



## AURORA A, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	<b>02-2068</b>
Quantity	5µg
Specific Activity	69 nmol/min/mg
Specific Lot Number	P071-1
Purity	>90%
Format	5µg in 50µl
Concentration	0.1 µg/µl
Stability	1yr At -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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## Activity Assay Protocol

### Reaction Components

#### Active Kinase (Catalog #: 02-2068)

Active AURORA A (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K21-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active AURORA A for optimal results).

#### Kinase Dilution Buffer, pH 7.2 (Catalog #: K21-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled .

#### Kinase Assay Buffer I, pH 7.2 (Catalog #: K01-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM , 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

#### [γ]-ATP Assay Cocktail

Prepare 250µM [γ]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [32P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at -20°C.

#### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200µl aliquots at -20°C.

#### Substrate

Myelin basic protein (MBP) diluted in distilled to a final concentration of 1mg/ml.

### Assay Protocol

- Step 1. Thaw [32P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active AURORA A, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  - Component 1. 10µl of diluted Active AURORA A (Catalog # 02-2068)
  - Component 2. 10µl of 1 mg/ml stock solution of substrate
- Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5. Initiate the reaction by the addition of 5µl [32P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

#### Calculation of [P<sup>32</sup>]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [32P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

#### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of 32P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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