

AURORA A, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # A28-18G

Lot # D097-1

Product Description

Recombinant full-length human AURORA A was expressed by baculovirus in Sf9 cells using an N-terminal GST tag. The gene accession number is [NM_003600](#).

Gene Aliases

AURKA, AIK, ARK1, AURA, BTAK, STK6, STK7, STK15, AURORA2, MGC34538

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 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.

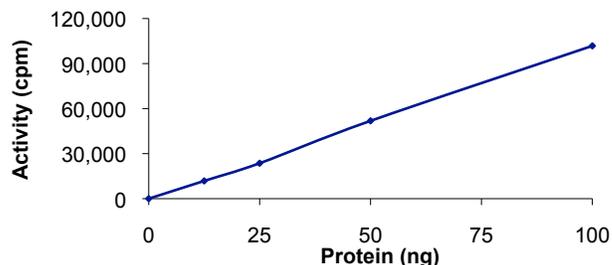
Scientific Background

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

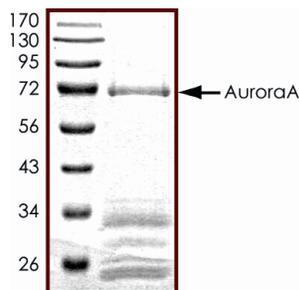
1. Dutertre, S. et al: On the role of aurora-A in centrosome function. *Oncogene*. 2002 Sep 9;21(40):6175-83.
2. Katayama, H. et al: The Aurora kinases: role in cell transformation and tumorigenesis. *Cancer Metastasis Rev*. 2003 Dec;22(4):451-64.

Specific Activity



The specific activity of AURORA A was determined to be **62 nmol /min/mg** as per activity assay protocol.

Purity



The purity of AURORA A was determined to be **>75%** by densitometry. Approx. MW **72kDa**.

AURORA A, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	A28-18G
Specific Activity	62 nmol/min/mg
Specific Lot Number	D097-1
Purity	>75%
Concentration	0.1 µg/µl
Stability	1 yr 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 #: A28-18G)

Active AURORA A (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

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

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (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 I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active AURORA A, Kinase Assay Buffer, Substrate and Kinase 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 #A28-18G)
 - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3. 5µl distilled H₂O (4°C)
- 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 H₂O.
- Step 5. Initiate the reaction by the addition of 5µl [³³P]-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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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