

Certificate of Analysis

Aurora B, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-835, 14-835-K, 14-835M

Parent Lot # D8PN002N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal 6His-tagged, recombinant, human Aurora B, full length and *N*-terminal GST-tagged, recombinant, human INCENP, amino acids 821-end. Co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Combined purity 84% by SDS-PAGE and Coomassie blue staining. Aurora B MW = 40kDa, INCENP MW = 38kDa

Specific Activity (Parent lot # D8PN002N): 734U/mg, where one unit of Aurora B activity is defined as 1nmol phosphate incorporated into 30 μ M (AKRRRLSSLRA) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.93mg/ml of 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

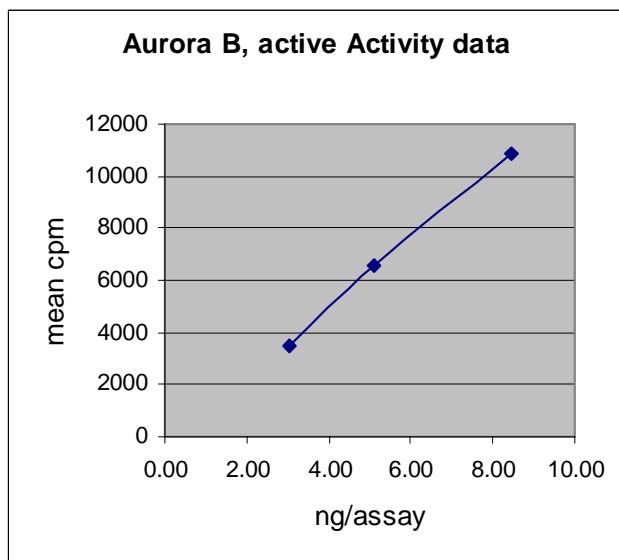
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

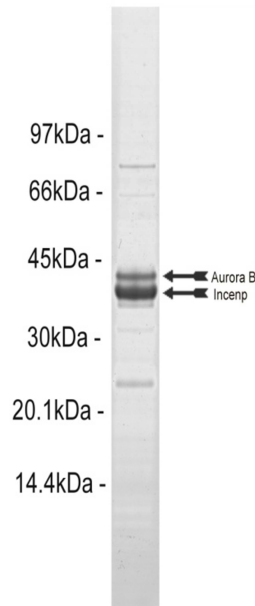
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3.06–8.44ng of this lot of enzyme phosphorylated 30 μ M (AKRRRLSSLRA) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as Aurora B with the translated sequence listed on page three. Confirmed identity as INCENP with the translated sequence listed on page four.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of active Aurora B.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA, other components.
2. **(AKRRRLSSLR):** Use at a final assay concentration of 30 μ M. Prepare a 0.3mM stock and add 2.5 μ l of stock per assay point.
3. **Aurora B, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3.06–8.44ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(AKRRRLSSLRA)**.
3. Add **2.5 μ l (3.06–8.44ng) Aurora B**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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Aurora B Sequence Information

<u>Protein</u>	human Aurora B
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the recombinant protein is equivalent to M1 of human Aurora B
<u>Accession number</u>	GenBank NM_004217

Recombinant Aurora B amino acid sequence:

```

1 MHHHHHMAQ KENSYPWPYG RQTAPSGLST LPQRVLRKEP VTPSALVLMs RSNVQPTAAP
61 GQKVMENSSG TPDILTRHFT IDDFEIGRPL GKGFVGNVYL AREKKSHFIV ALKVLFKSQI
121 EKEGVEHQLR REIEIQAHLH HPNILRLYNY FYDRRRRIYLI LEYAPRGELY KELQKSCFTD
181 EQRTATIMEE LADALMYCHG KKVIHRDIKP ENLLLGLKGE LKIADFGWSV HAPSLRRKTM
241 CGTLDYLPPE MIEGRMHNEK VDLWCIGVLC YELLVGNPPF ESASHNETYR RIVKVDLKFV
301 ASVPTGAQDL ISKLLRHNSP ERLPLAQVSA HPWVRANSRR VLPPSALQSV A

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Recombinant Aurora B nucleotide sequence:

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1 atgcaccatc accatcacca tatggcccag aaggagaact cctaccctg gccctacggc
61 cgacagacgg ctccatctgg cctgagcacc ctgccccagc gagtcctccg gaaagagcct
121 gtcaccccat ctgcacttgt cctcatgagc cgctccaatg tccagcccac agctgcccct
181 gcccagaagg tgatggagaa tagcagtggg acacccgaca tcttaacgcg gcacttcaca
241 attgatgact ttgagattgg gcgtcctctg ggcaaaggca agtttgaaa cgtgtacttg
301 gctcgggaga agaaaagcca ttcatcgtg gcgctcaagg tcctcttcaa gtcccagata
361 gagaaggagg gcgtggagca tcagctgctc agagagatcg aaatccaggc ccacctgcac
421 catccaaca tcctgctctc ctacaactat tttatgacc ggaggaggat ctacttgatt
481 ctgagatgat cccccgcgg ggagctctac aaggagctgc agaagagctg cacatttgac
541 gagcagcgaa cagccacgat catggaggag ttggcagatg ctctaata gta ctgccatggg
601 aagaagggtg ttcacagaga cataaagcca gaaaatctgc tcttagggct caaggagag
661 ctgaagattg ctgacttcgg ctggctctgt catgctgccc cctgaggag gaagacaatg
721 tgtggcacc tggactacct gccccagag atgattgagg ggcgatgca caatgagaag
781 gtggatctgt ggtgcattgg agtgctttgc tatgagctgc tgggtgggaa cccacccttt
841 gagagtgcac cacacaacga gacctatcgc cgcacgtca aggtggacct aaagtcccc
901 gcttctgtgc ccacgggagc ccaggacctc atctccaaac tgctcaggca taaccctcg
961 gaacggctgc ccctggcca ggtctcagcc caccctggg tccgggcaa ctctcggagg
1021 gtgctgcctc cctctgcctc tcaatctgtc gcctga

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INCENP Sequence Information

<u>Protein</u>	human INCENP
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	D231 of the recombinant protein is equivalent to D821 of human INCENP
<u>Accession number</u>	GenBank NM_020238

Recombinant INCENP amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQ SMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAI PQID  KYLKSSKYIA  WPLQGWQATF  GGDHPPKSD  LEVLFQGPEF  DLNSDDSTDD
241  EAHPRK PIPT  WARGTPLSQA  IIHQYYHPPN  LLELFGTILP  LDLEDIFKKS  KPRYHKRTSS
301  AVWNSP PLQG  ARVPSSLAYS  LKKH

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Recombinant INCENP nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatata  ttgaagaaaa  atatgaagag  catttgatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gaggtttcca  atcttcctta  ttatattgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacgtt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcgggtttg
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361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatttaa  atggtgatca  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggacc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggaagtcc  tgttccaggg  gccgaattc  gatctgaata  gcgacgactc  caccgatgat
721  gaggcccatc  cccggaagcc  catccccacc  tgggcccag  gcaccccgct  cagccaggct
781  atcattcacc  agtactacca  cccaccgaac  cttctggagc  tctttggaac  catttcccca
841  ctggacttgg  aggatatctt  caagaagagc  aagccccgct  atcacaagcg  caccagctct
901  gctgtctgga  actcaccgcc  cctgcagggc  gccagggctc  ccagcagcct  ggcctacagc
961  ctgaagaagc  actga

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Reviewed and approved by site quality representative.

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