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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, active activity is defined as 1nmol phosphate incorporated into 30µM (AKRRRLSSLRA) per minute at 30°C with a final ATP concentration of 100µM.

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.

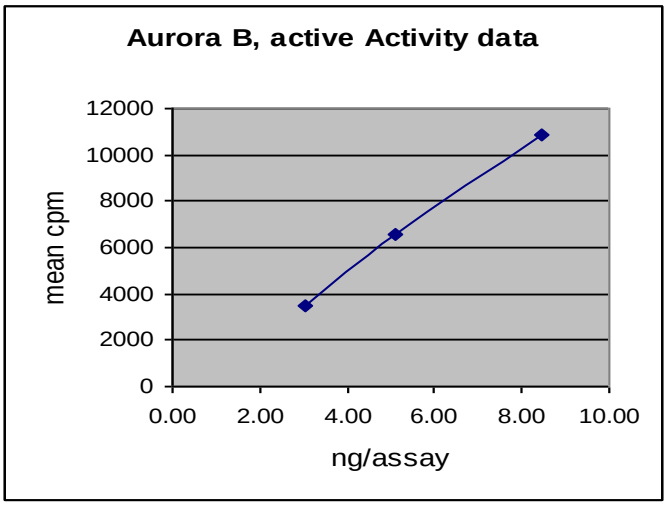
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

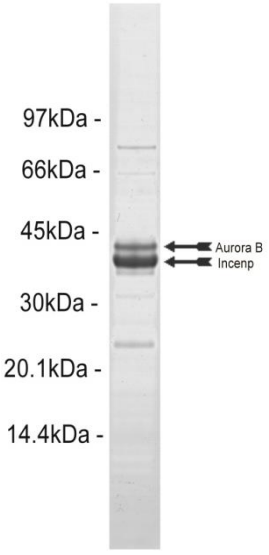
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 3.06–8.44ng of this lot of enzyme phosphorylated 30µM (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 3µg of Aurora B, active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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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 30µM. Prepare a 0.3mM stock and add 2.5µl 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-^{33}P]ATP:** 2.5 x MgAc/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(AKRRRLSSLRA)**.
3. Add **2.5µl (3.06–8.44ng) Aurora B**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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Aurora B Sequence Information

Protein human Aurora B

Tags *N*-terminal 6His

Native sequence M8 of the recombinant protein is equivalent to M1 of human Aurora B

Accession number GenBank NM_004217

Recombinant Aurora B amino acid sequence:

```
   1 MHHHHHHMAQ KENSYPWPYG RQTAPSGLST LPQRVLRKEP VTPSALVLMS RSNVQPTAAP
  61 GQKVMENSSG TPDILTRHFT IDDFEIGRPL GKGKFGNVYL AREKKSHFIV ALKVLFKSQI
 121 EKEGVEHQLR REIEIQAHLH HPNILRLYNY FYDRRRIYLI LEYAPRGELY KELQKSCTFD
 181 EQRTATIMEE LADALMYCHG KKVIHRDIKP ENLLLGLKGE LKIADFGWSV HAPSLRRKTM
 241 CGTLDYLPPE MIEGRMHNEK VDLWCIGVLC YELLVGNPPF ESASHNETYR RIVKVDLKFP
 301 ASVPTGAQDL ISKLLRHNPS ERLPLAQVSA HPWVRANSRR VLPPSALQSV A
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Recombinant Aurora B nucleotide sequence:

```
   1 atgcaccatc accatcacca tatggcccag aaggagaact cctacccctg gccctacggc
  61 cgacagacgg ctccatctgg cctgagcacc ctgccccagc gagtcctccg gaaagagcct
 121 gtcaccccat ctgcacttgt cctcatgagc cgctccaatg tccagcccac agctgccccc
 181 ggccagaagg tgatggagaa tagcagtggg acacccgaca tcttaacgcg gcacttcaca
 241 attgatgact ttgagattgg gcgtcctctg ggcaaaggca agtttggaaa cgtgtacttg
 301 gctcgggaga agaaaagcca tttcatcgtg gcgctcaagg tcctcttcaa gtcccagata
 361 gagaaggagg gcgtggagca tcagctgcgc agagagatcg aaatccaggc ccacctgcac
 421 catcccaaca tcctgcgtct ctacaactat ttttatgacc ggaggaggat ctacttgatt
 481 ctagagtatg ccccccgcgg ggagctctac aaggagctgc agaagagctg cacatttgac
 541 gagcagcgaa cagccacgat catggaggag ttggcagatg ctctaatgta ctgccatggg
 601 aagaaggtga ttcacagaga cataaagcca gaaaatctgc tcttagggct caagggagag
 661 ctgaagattg ctgacttcgg ctggtctgtg catgcgccct ccctgaggag gaagacaatg
 721 tgtggcaccc tggactacct gcccccagag atgattgagg ggcgcatgca caatgagaag
 781 gtggatctgt ggtgcattgg agtgctttgc tatgagctgc tggtggggaa cccacccttt
 841 gagagtgcat cacacaacga gacctatcgc cgcatcgtca aggtggacct aaagttcccc
 901 gcttctgtgc ccacgggagc ccaggacctc atctccaaac tgctcaggca taacccctcg
 961 gaacggctgc ccctggccca ggtctcagcc cacccttggg tccgggccaa ctctcggagg
1021 gtgctgcctc cctctgccct tcaatctgtc gcctga
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INCENP Sequence Information

Protein human INCENP

Tags *N*-terminal GST

Native sequence D231 of the recombinant protein is equivalent to D821 of human INCENP

Accession number GenBank NM_020238

Recombinant INCENP amino acid sequence:

```
  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF DLNSDDSTDD
241 EAHPRKPIPT WARGTPLSQA IIHQYYHPPN LLELFGTILP LDLEDIFKKS KPRYHKRTSS
301 AVWNSPPLQG ARVPSSLAYS LKKH
```

Recombinant INCENP nucleotide sequence:

```
  1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
 61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggaagttc tgttccaggg gcccgaattc gatctgaata gcgacgactc caccgatgat
721 gaggcccatc cccggaagcc catccccacc tgggcccgag gcaccccgct cagccaggct
781 atcattcacc agtactacca cccaccgaac cttctggagc tctttggaac cattctccca
841 ctggacttgg aggatatctt caagaagagc aagccccgct atcacaagcg caccagctct
901 gctgtctgga actcaccgcc cctgcagggc gccagggtcc ccagcagcct ggcctacagc
961 ctgaagaagc actga
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Reviewed and approved by site quality representative.