

Certificate of Analysis

Aurora C, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-911, 14-911-K, 14-911M

Parent Lot # D10JP007N

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 C amino acids 35-end and N-terminal GST-tagged, recombinant, human INCENP, amino acids 821-end. Co-expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose.

Combined purity 94% by SDS-PAGE and Coomassie blue staining. Aurora C MW = 36kDa, INCENP MW = 38kDa.

Specific Activity (Parent lot# D10JP007N):

2057U/mg, where one unit of Aurora C, active activity is defined as 1nmol phosphate incorporated into 30μM (AKRRRLSSLRA) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.43mg/ml of enzyme in 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.

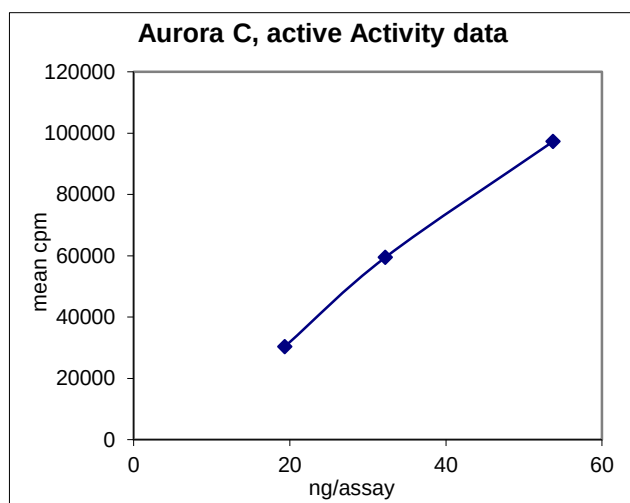
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

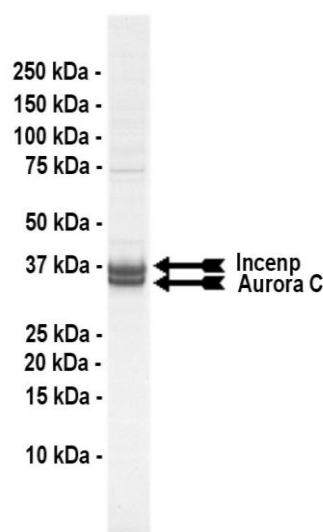
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4–54ng 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 C 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 C, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **10% Triton X-100:** Use at a final assay concentration of 0.01%. Add 0.25µl of a 10% solution in water.
3. **Sodium Chloride:** Use at a final assay concentration of 50mM. Add 0.42µl of a 3M solution.
4. **(AKRRRLSSLRA):** Use at a final assay concentration of 30µM. Prepare a 300µM stock and add 2.5µl of stock per assay point.
5. **Aurora C, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4–54ng per assay point
6. **[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 500-800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 0.25µl of 10% Triton X-100
3. Add 0.42µl of 3M sodium chloride
4. Add 2.5µl of **(AKRRRLSSLRA)**.
5. Add **2.5µl (4–54ng) Aurora C, active**.
6. Add 4.33µl of dH₂O.
7. Add 10µl of diluted [γ-³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl aliquot onto the appropriate area of a P30 Filtermat.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. 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 C Sequence Information

<u>Protein</u>	human Aurora C
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M35 of human Aurora C
<u>Accession number</u>	GenBank AB017332.1

Recombinant Aurora C amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMSLGS MRRLTVDDFE IGRPLGKGF GNVYLARLKE
61 SHFIVALKVL FKSQIEKEGL EHQLRREIEI QAHLQHPNIL RLYNYFHDAR RYVLILEYAP
121 RGELYKELQK SEKLDEQRTA TIIIEELADAL TYCHDKKVIH RDIKPENLLL GFRGEVKIAD
181 FGWSVHTPSL RRTMCGTLD YLPPEMIEGR TYDEKVDLWC IGVLCYELLV GYPPEFESASH
241 SETYRRILKV DVRFPLSMPL GARDLISRL RYQPLERLPL AQILKHPWVQ AHSRRVLPPC
301 AQMAS

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Recombinant Aurora C nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgtc ccttggatcc atgcggcgcc tcacagtcga tgactttgaa
121 atcgggctgc ccctgggcaa ggggaaattt gggaatgtgt acctggctcg gctcaaggaa
181 agccatttca ttgtggccct gaaggttctc ttcaagtcgc agatagagaa ggaaggactg
241 gagcaccagc tgcgccggga aattgagatc caggctcatc tacaacaccc caatattcctg
301 cgctgtata actatttcca tgatgcacgc cgggtgtacc tgattctgga atatgctcca
361 aggggtgagc tctacaagga gctgcagaaa agcgagaaat tagatgaaca gcgcacagcc
421 acgataatag aggagttggc agatgccctg acctactgcc atgacaagaa agtgattcac
481 agagatatta agccagagaa cctgctgctg gggttcaggg gtgagggtgaa gattgcagat
541 tttggctggt ctgtgcacac cccctccctg aggaggaaga caatgtgtgg gacactggac
601 tacttgccgc cagaaatgat tgaggggaga acatatgatg aaaaggtgga tttgtggtgc
661 attggagtgc tctgctatga gctgctggtg ggatatccac ctttgagag cgctccccc
721 agtgagactt acagacgcat cctcaaggta gatgtgaggt ttccactatc aatgcctctg
781 ggggcccggg acttgatttc caggcttctc agataccagc ctttgagag actgcccctg
841 gccagatcc tgaagcacc ctgggttcag gccactccc gaagggtgct gcctccctgt
901 gctcagatgg cttcctag

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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_020238.2

Recombinant INCENP amino acid sequence:

```

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

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Recombinant INCENP nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatattgat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatagctt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
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361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatataa  atggtgatca  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggaagtgc  tgttccaggg  gcccgaattc  gatctgaata  ggcacgactc  caccgatgat
721  gaggcccatc  cccggaagcc  catccccacc  tgggcccag  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.

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