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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 Ni^{2+}/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.

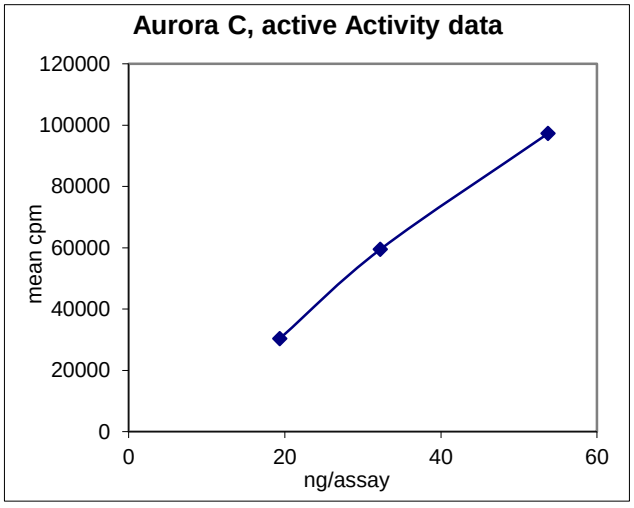
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

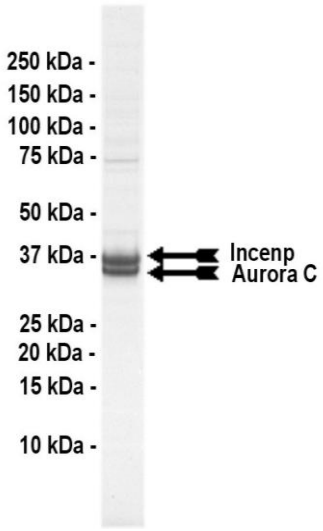
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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-^{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 500-800 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 dH_2O.
7. Add 10µl of diluted [γ-^{33}P]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

Protein human Aurora C

Tags *N*-terminal 6His

Native sequence M31 of the recombinant protein is equivalent to M35 of human Aurora C

Accession number GenBank AB017332.1

Recombinant Aurora C amino acid sequence:

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMSLGS MRRLTVDDFE IGRPLGKGKF GNVYLARLKE
 61 SHFIVALKVL FKSQIEKEGL EHQLRREIEI QAHLQHPNIL RLYNYFHDAR RVYLILEYAP
121 RGELYKELQK SEKLDEQRTA TIIEELADAL TYCHDKKVIH RDIKPENLLL GFRGEVKIAD
181 FGWSVHTPSL RRKTMCGTLD YLPPEMIEGR TYDEKVDLWC IGVLCYELLV GYPPFESASH
241 SETYRRILKV DVRFPLSMPL GARDLISRLL RYQPLERLPL AQILKHPWVQ AHSRRVLPPC
301 AQMAS
```

Recombinant Aurora C nucleotide sequence:

```
  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatgtc ccttggatcc atgcggcgcc tcacagtcga tgactttgaa
121 atcgggcgtc ccctgggcaa ggggaaattt gggaatgtgt acctggctcg gctcaaggaa
181 agccatttca ttgtggccct gaaggttctc ttcaagtcgc agatagagaa ggaaggactg
241 gagcaccagc tgcgccggga aattgagatc caggctcatc tacaacaccc caatatcctg
301 cgcctgtata actatttcca tgatgcacgc cgggtgtacc tgattctgga atatgctcca
361 aggggtgagc tctacaagga gctgcagaaa agcgagaaat tagatgaaca gcgcacagcc
421 acgataatag aggagttggc agatgccctg acctactgcc atgacaagaa agtgattcac
481 agagatatta agccagagaa cctgctgctg gggttcaggg gtgaggtgaa gattgcagat
541 tttggctggt ctgtgcacac cccctccctg aggaggaaga caatgtgtgg gacactggac
601 tacttgccgc cagaaatgat tgaggggaga acatatgatg aaaaggtgga tttgtggtgc
661 attggagtgc tctgctatga gctgctggtg ggatatccac cctttgagag cgcctcccac
721 agtgagactt acagacgcat cctcaaggta gatgtgaggt ttccactatc aatgcctctg
781 ggggcccggg acttgatttc caggcttctc agataccagc ccttggagag actgcccctg
841 gcccagatcc tgaagcaccc ctgggttcag gcccactccc gaagggtgct gcctccctgt
901 gctcagatgg cttcctag
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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_020238.2

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.