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# Certificate of Analysis

## Aurora A, active

**(Recombinant enzyme expressed in Sf21 insect cells.)**
**Item # 14-511, 14-511-K, 14-511M**
**Parent Lot # 1623025**

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 full-length human Aurora-A, expressed by baculovirus in Sf21 insect cells. Purified using $Ni^{2+}$/NTA agarose. Autoactivated by incubating with Mg/ATP and redialysed to remove excess ATP. Purity 94% by SDS-PAGE and Coomassie blue staining.
MW = 46.9kDa.

**Specific Activity (Parent lot# 1623025):** 1598U/mg, where one unit of Aurora-A activity is defined as 1nmol phosphate incorporated into 200µM Kemptide (LRRASLG) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation: 0.289mg/ml** of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

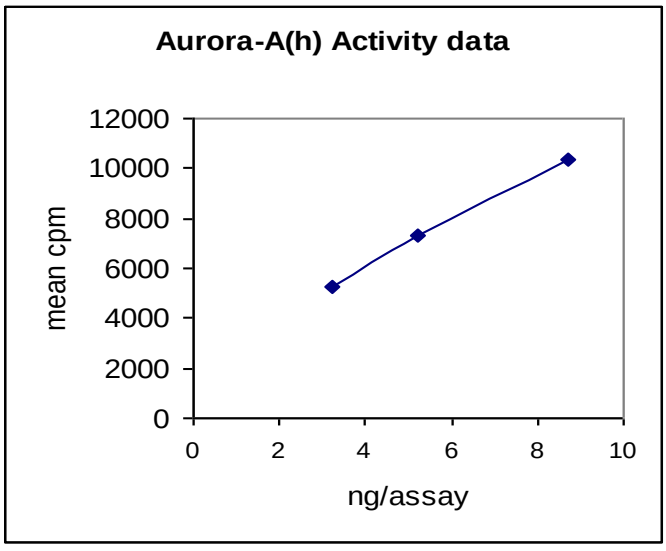
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the 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.

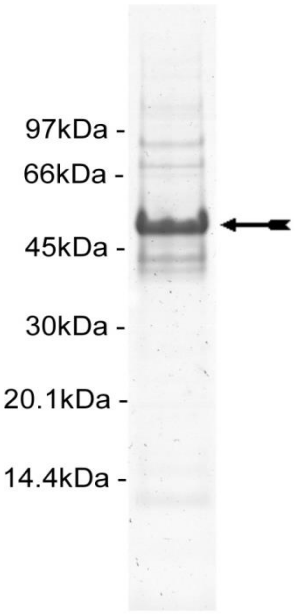
**FOR IN VITRO RESEARCH USE ONLY**
**NOT FOR USE IN HUMANS OR ANIMALS**

## Quality Control Testing

Kinase Assay: 3.2–8.7ng of this lot of enzyme phosphorylated 200µM Kemptide 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 product identity as Aurora-A with the translated sequence listed on page three.




SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 µg of Aurora-A.

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## Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Kemptide:** Use at a final assay concentration of 200µM. Prepare a 2mM stock. Add 2.5µl of stock per assay point.
3. **Aurora-A, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3.2–8.7ng per assay point.
4. **[γ-$^{33}$P]ATP:** 2.5 x magnesium acetate/[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 **kemptide**.
3. Add **2.5µl (3.2–8.7ng) Aurora-A, active**.
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-A Sequence Information

**Protein** human Aurora-A

**Tags** *N*-terminal 6His

**Native sequence** M8 of the recombinant protein is equivalent to M1 of human Aurora-A

**Accession number** GenBank NM_003600. The recombinant protein also contains the conflict I31F with respect to GenBank NM_003600. This substitution is reported in GenBank NM_003158 and BC027464. The residue coordinates in the native sequence are given.

***Recombinant Aurora-A amino acid sequence:***

```
  1 MHHHHHHMDR SKENCISGPV KATAPVGGPK RVLVTQQFPC QNPLPVNSGQ AQRVLCPSNS
 61 SQRVPLQAQK LVSSHKPVQN QKQKQLQATS VPHPVSRPLN NTQKSKQPLP SAPENNPEEE
121 LASKQKNEES KKRQWALEDF EIGRPLGKGK FGNVYLAREK QSKFILALKV LFKAQLEKAG
181 VEHQLRREVE IQSHLRHPNI LRLYGYFHDA TRVYLILEYA PLGTVYRELQ KLSKFDEQRT
241 ATYITELANA LSYCHSKRVI HRDIKPENLL LGSAGELKIA DFGWSVHAPS SRRTTLCGTL
301 DYLPPEMIEG RMHDEKVDLW SLGVLCYEFL VGKPPFEANT YQETYKRISR VEFTFPDFVT
361 EGARDLISRL LKHNPSQRPM LREVLEHPWI TANSSKPSNC QNKESASKQS
```

***Recombinant Aurora-A nucleotide sequence:***

```
   1 atgcatcacc atcaccatca tatggaccga tctaaagaaa actgcatttc aggacctgtt
  61 aaggctacag ctccagttgg aggtccaaaa cgtgttctcg tgactcagca atttccttgt
 121 cagaatccat tacctgtaaa tagtggccag gctcagcggg tcttgtgtcc ttcaaattct
 181 tcccagcgcg ttcctttgca agcacaaaag cttgtctcca gtcacaagcc ggttcagaat
 241 cagaagcaga agcaattgca ggcaaccagt gtacctcatc ctgtctccag gccactgaat
 301 aacacccaaa agagcaagca gcccctgcca tcggcacctg aaaataatcc tgaggaggaa
 361 ctggcatcaa aacagaaaaa tgaagaatca aaaaagaggc agtgggcttt ggaagacttt
 421 gaaattggtc gccctctggg taaaggaaag tttggtaatg tttatttggc aagagaaaag
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 541 gtggagcatc agctcagaag agaagtagaa atacagtccc accttcggca tcctaatatt
 601 cttagactgt atggttattt ccatgatgct accagagtct acctaattct ggaatatgca
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 721 gctacttata taacagaatt ggcaaatgcc ctgtcttact gtcattcgaa gagagttatt
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 961 agccttggag ttctttgcta tgaattttta gttgggaagc ctccttttga ggcaaacaca
1021 taccaagaga cctacaaaag aatatcacgg gttgaattca cattccctga ctttgtaaca
1081 gagggagcca gggacctcat ttcaagactg ttgaagcata atcccagcca gaggccaatg
1141 ctcagagaag tacttgaaca cccctggatc acagcaaatt catcaaaacc atcaaattgc
1201 caaaacaaag aatcagctag caaacagtct tag
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Reviewed and approved by site quality representative.