

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Aurora A [2 – 403]

Enzyme description:- Aurora A [2 - 403]

Clone number:- DU 4174

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

Purification method:- Ni²⁺-NTA agarose

Expression level:- 4 mg/L

Calculated molecular mass:-

Monoisotopic 49, 191.32 daltons

Average Mass 49, 222.02 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.51

Purity:- >80 %

Activation protocol:-

Autotactivated by incubation with Mg/ATP and redialysed to remove excess ATP

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

LRRLSLGLRRLSLGLRRLSLGLRRLSLG Final concentration: 300 μM

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Clone Data Sheet

Aurora A [2 - 403]

Protein Aurora A [2 – 403]

Clone number DU 4174

Species Human

Accession number BC027464

Tags N-terminal His(6)

Bacterial expressed protein MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSDRSKENCISGPVKAT
APVGGPKRVLVTQQFPCQNPLPVNSGQAQRVLCPSNSSQRVPLQAQKLV
SSHKPVQNKQKQLQATSVPHPVSRPLNNTQKSKQPLPSAPENNPEEEL
ASKQNEESKKRQWALEDFEIGRPLGKGFGNVYLAREQOSKFILALKV
LFKAQLEKAGVEHQLRREVEIQSHLRHPNILRLYGYFHDATRVYLILEY
APLGTVYRELOKLSKFDEQRTATYITELANALSYCHSKRVIHRDIKPEN
LLGSAGELKIADFGWSVHAPSSRRTTLCGTLDYLPPEMIEGRMHDEKV
DLWSLGVLCYEFLVGKPPFEANTYQETYKRISRVEFTFPDFVTEGARDL
ISRLLKHNPSQRPMLREVLEHPWITANSSKPSNCONKESASKQS

Native sequence Amino acids D2 – S403 (end) of human Aurora A.
Residue D35 of the fusion protein is equivalent to D2 of the native enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage Thrombin (LVPRGS) at residues 14 - 19

Cloning sites *Bam*H1 and *Sal*1 sites of pET28a(+)

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**Complete
nucleotide
Sequence**

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGC
GCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTTCGCGG
ATCCGACCGATCTAAAGAAAACGATTTTCAGGACCTGTAAAGGCTACA
GCTCCAGTTGGAGGTCCAAAACGTGTTCTCGTGAAGTCAAGCAATTTCCCTT
GTCAGAATCCATTACCTGTAAATAGTGGCCAGGCTCAGCGGGTCTTGTC
TCCTTCAAATTTCTTCCCAGCGCGTTCCTTTGCAAGCACAAAAGCTTGTC
TCCAGTCACAAGCCGGTTCAGAATCAGAAGCAGAAGCAATTGCAGGCAA
CCAGTGTACCTCATCCTGTCTCCAGGCCACTGAATAACACCCAAAAGAG
CAAGCAGCCCCTGCCATCGGCACCTGAAAATAATCCTGAGGAGGAACTG
GCATCAAAACAGAAAAATGAAGAATCAAAAAAGAGGCAGTGGGCTTTGG
AAGACTTTGAAATTTGGTCGCCCTCTGGGTAAAGGAAAGTTTGGTAATGT
TTATTTGGCAAGAGAACAGCAAAGCAAGTTTATTCTGGCTCTTAAAGTG
TTATTTAAAGCTCAGCTGGAGAAAGCCGGAGTGGAGCATCAGCTCAGAA
GAGAAGTAGAAATACAGTCCCACCTTCGGCATCCTAATATTCTTAGACT
GTATGGTTATTTCCATGATGCTACCAGAGTCTACCTAATTTCTGGAATAT
GCACCACTTGGAACAGTTTATAGAGAACTTCAGAACTTTCAAAGTTTG
ATGAGCAGAGAAGTCTACTTATATAACAGAATTGGCAAATGCCCTGTC
TTACTGTCATTCGAAGAGAGTTATTCATAGAGACATTAAGCCAGAGAAC
TTACTTCTTGATCAGCTGGAGAGCTTAAAATTGCAGATTTTGGGTGGT
CAGTACATGCTCCATCTTCCAGGAGGACCCTCTCTGTGGCACCCCTGGA
CTACCTGCCCCCTGAAATGATTGAAGGTCGGATGCATGATGAGAAGGTG
GATCTCTGGAGCCTTGGAGTTCTTTGCTATGAATTTTTAGTTGGGAAGC
CTCCTTTTGAGGCAAACACATAACCAAGAGACCTACAAAAGAATATCACG
GGTTGAATTCACATTCCTGACTTTGTAAACAGAGGGAGCCAGGGACCTC
ATTTCAAGACTGTTGAAGCATAATCCCAGCCAGAGGCCAATGCTCAGAG
AAGTACTTGAACACCCCTGGATCACAGCAAATTCATCAAAACCATCAAA
TTGCCAAAACAAAGAATCAGCTAGCAAACAGTCTtaggtcgac