

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 [Long term stability to be determined]

Assay:- Standard filter binding assay

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

Specific activity range:- To be determined

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Clone Data Sheet

Aurora A [2 - 403]

<u>Protein</u>	Aurora A [2 – 403]
<u>Clone number</u>	DU 4174
<u>Species</u>	Human
<u>Accession number</u>	BC027464
<u>Tags</u>	N-terminal His(6)
<u>Bacterial expressed protein</u>	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSDRSKENCISGPVKAT APVGGPKRVLVTQQFPCQNPLPVNSGQAQRVLCPSNSSQRVPLQAQKLV SSHKPVQNQKQKQLQATSVPHPVSRPLNNTQKSKQPLPSAPENNPEEEL ASKQKNEESKKRQWALEDFEIGRPLGKGKFGNVYLAREQQSKFILALKV LFKAQLEKAGVEHQLRREVEIQSHLRHPNILRLYGYPHDATRVYLILEY APLGTVYRELQKLSKFDEQRTATYITELANALSYCHSKRVIHRDIKPEN LLGSAGELKIADFGWSVHAPSSRRTTLCGTLDYLPPEMIEGRMHDEKV DLWSLGVLCYEFLVGKPPFEANTYQETYKRISRVEFTFPDFVTEGARDL ISRLLKHNPSSQRPMLREVLEHPWITANSSKPSNCQNKESASKQS
<u>Native sequence</u>	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.
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) at residues 14 - 19
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Sal</i> I sites of pET28a(+)

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Complete nucleotide Sequence

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGC
GCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTTCGCGG
ATCCGACCGATCTAAAGAAAACATGCAATTCAGGACCTGTTAAGGCTACA
GCTCCAGTTGGAGGTCCAAAACGTGTTCTCGTGAAGTCAAGCAATTCCTT
GTCAGAATCCATTACCTGTAAATAGTGGCCAGGCTCAGCGGGTCTTGTC
TCCTTCAAATTCTTCCCAGCGCGTTCCTTTGCAAGCACAAAAGCTTGTC
TCCAGTCACAAGCCGGTTCAGAATCAGAAGCAGAAGCAATTGCAGGCAA
CCAGTGTACCTCATCCTGTCTCCAGGCCACTGAATAACACCCAAAAGAG
CAAGCAGCCCCTGCCATCGGCACCTGAAAATAATCCTGAGGAGGAACTG
GCATCAAAACAGAAAAATGAAGAATCAAAAAAGAGGCAGTGGGCTTTGG
AAGACTTTGAAATTGGTCGCCCTCTGGGTAAAGGAAAGTTTGTAATGT
TTATTTGGCAAGAGAACAGCAAAGCAAGTTTATTCTGGCTCTTAAAGTG
TTATTTAAAGCTCAGCTGGAGAAAGCCGGAGTGGAGCATCAGCTCAGAA
GAGAAGTAGAAATACAGTCCCACCTTCGGCATCCTAATATTCTTAGACT
GTATGGTTATTTCCATGATGCTACCAGAGTCTACCTAATTCTGGAATAT
GCACCACTTGGAACAGTTTATAGAGAACTTCAGAACTTTCAAAGTTTG
ATGAGCAGAGAACTGCTACTTATATAACAGAATTGGCAAATGCCCTGTC
TTACTGTCATTTCGAAGAGAGTTATTCATAGAGACATTAAGCCAGAGAAC
TTACTTCTTGGATCAGCTGGAGAGCTTAAAATTGCAGATTTTGGGTGGT
CAGTACATGCTCCATCTTCCAGGAGGACCACTCTCTGTGGCACCCCTGGA
CTACCTGCCCCCTGAAATGATTGAAGGTCGGATGCATGATGAGAAGGTG
GATCTCTGGAGCCTTGGAGTTCTTTGCTATGAATTTTTAGTTGGGAAGC
CTCCTTTTGAGGCAAACACATACCAAGAGACCTACAAAAGAATATCACG
GGTTGAATTCACATTCCTGACTTTGTAAACAGAGGGAGCCAGGGACCTC
ATTTCAAGACTGTTGAAGCATAATCCCAGCCAGAGGCCAATGCTCAGAG
AAGTACTTGAACACCCCTGGATCACAGCAAATTCATCAAAACCATCAAA
TTGCCAAAACAAAGAATCAGCTAGCAAACAGTCTtaggtcgac