Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Aurora B [1 - 344]

Enzyme description:- Aurora B [1 - 344]

Clone number:- DU 1773

Source:- Recombinant

Expression system:- Baculovirus expression vector system
Following expression the culture is incubated with
50 nM okadaic acid for 1 hour prior to purification

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic	40, 208.97 daltons
Average Mass	40, 234.49 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 9.36

Purity:- >80 %

Activation protocol:-

Does not require Incenp for activity if assayed against the tetra (LRRLSLG) substrate peptide

Enzyme storage buffer:-

50 mM Tris-HCl pH 8, 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]

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Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2−mercaptoethanol, 0.1 mM EGTA,
0.1 mM sodium vanadate, 10 mM magnesium acetate

Substrate:-
LRRLSLGLRRLSLGLRRLSLGLRRLSLG Final concentration: 300 µM

Specific activity range:- To be determined

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Clone Data Sheet

Aurora B [1 - 344]

Protein	Aurora B [1 – 344]
Clone number	DU 1773
Species	Human
Accession number	NM_004217
Tags	N-terminal His(6)
Baculovirus expressed protein	MHHHHHH**MAQKENSYPWPYGRQTAPSGLSTLPQRVLRKEPVTPSAL** **VLMSRSNVQPTAAPGQKVMENSSGTPDILTRHFTIDDFEIGRPLGK** **GKFGNVYLAREKKSHFIVALKVLFKSQIEKEGVEHQLRREIEIQAH** **LHHPNILRLYNYFYDRRRIYLILEYAPRGELYKELQKSCTFDEQRT** **ATIMEELADALMYCHGKKVIHRDIKPENLLLGLKGELKIADFGWSV** **HAPSLRRKTMCGTLDYLPPEMIEGRMHNEKVDLWCIGVLCYELLVG** **NPPFESASHNETYRRIVKVDLKFPASVPTGAQDLISKLLRHNPSER** **LPLAQVSAHPWVRANSRRVLPPSALQSVA**
Native sequence	Amino acids M1 – A344 (end) of human Aurora B. Residue M8 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 2 – 7.
Protease cleavage	None
Cloning sites	*Nde*1 and *Xho*1 sites of modified pFastBAC 1

Complete nucleotide Sequence	ATGCACCATCACCATCACCATATGGCCCAGAAGGAGAACTCCTA CCCCTGGCCCTACGGCCGACAGACGGCTCCATCTGGCCTGAGCA CCCTGCCCCAGCGAGTCCTCCGGAAAGAGCCTGTCACCCCATCT GCACTTGTCCTCATGAGCCGCTCCAATGTCCAGCCCACAGCTGC CCCTGGCCAGAAGGTGATGGAGAATAGCAGTGGGACACCCGACA TCTTAACGCGGCACTTCACAATTGATGACTTTGAGATTGGGCGT CCTCTGGGCAAAGGCAAGTTTGGAAACGTGTACTTGGCTCGGGA GAAGAAAAGCCATTTCATCGTGGCGCTCAAGGTCCTCTTCAAGT CCCAGATAGAGAAGGAGGGCGTGGAGCATCAGCTGCGCAGAGAG ATCGAAATCCAGGCCCACCTGCACCATCCCAACATCCTGCGTCT CTACAACTATTTTTATGACCGGAGGAGGATCTACTTGATTCTAG AGTATGCCCCCCGCGGGGAGCTCTACAAGGAGCTGCAGAAGAGC TGCACATTTGACGAGCAGCGAACAGCCACGATCATGGAGGAGTT GGCAGATGCTCTAATGTACTGCCATGGGAAGAAGGTGATTCACA GAGACATAAAGCCAGAAAATCTGCTCTTAGGGCTCAAGGGAGAG CTGAAGATTGCTGACTTCGGCTGGTCTGTGCATGCGCCCTCCCT GAGGAGGAAGACAATGTGTGGCACCCTGGACTACCTGCCCCCAG AGATGATTGAGGGGCGCATGCACAATGAGAAGGTGGATCTGTGG TGCATTGGAGTGCTTTGCTATGAGCTGCTGGTGGGGAACCCACC CTTTGAGAGTGCATCACACAACGAGACCTATCGCCGCATCGTCA AGGTGGACCTAAAGTTCCCCGCTTCTGTGCCCACGGGAGCCCAG GACCTCATCTCCAAACTGCTCAGGCATAACCCCTCGGAACGGCT GCCCCTGGCCCAGGTCTCAGCCCACCCTTGGGTCCGGGCCAACT CTCGGAGGGTGCTGCCTCCCTCTGCCCTTCAATCTGTCGCCtga