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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