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

Tag:- N-terminal His(6)

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

Expression level:- 2 mg/L

Calculated molecular mass:- 40, 208 daltons

Purity:- >80 %

Activation protocol:-

Aurora B [5 μM] is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.1 mM sodium vandate, 10 mM magnesium acetate, 0.1 mM ATP with 0.1 mg/ml GST-Incenp [826 - 919] at 30 °C for 30 mins. Following activation the enzyme is then dialysed into enzyme storage buffer and stored at –70 °C.

Note: as the Incenp fragment binds to Aurora B it cannot be removed from the preparation.

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

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:-
RRRLSFAEPG Final concentration: 300 μM

Specific activity range:- 75 - 150 U/mg

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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

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Incenp [826 - 919]

Enzyme description:- Incenp [826 - 919]

Clone Number:- DU 930

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 0.5 mg/L

Calculated molecular mass:- 37, 463 daltons

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

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

Storage temperature:- -20 °C.

Division of Signal Transduction Therapy

Clone Data Sheet

Incenp [826 - 919]

Protein Incenp [826 – 919]

Clone number DU 930

Species Human

Accession number NM_020238

Tags N-terminal GST

E. coli expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDK
WRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHN
MLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKV
DFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALD
VVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA
WPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**DLNSDDSTD**
DEAHPRKPIPTWARGTPLSQAIIHQYYQPPNLLELFGTIL
PLDLEDIFKKSKPRYHKRTSSAVWNSPPLQGARVPSSLAY
SLKKH

Native sequence Amino acids D826 – H919 (end) of human Incenp.
Residue D232 of the fusion protein is equivalent to D826 of the native enzyme. The GST tag is located at residues 1 - 220

Protease cleavage PreScission (LEVLFQGPL) at residues 221 - 229

Cloning sites *Bam* H1 and *Eco* R1 of pGEX6P-1

Nucleotide sequence of insert

GATCTGAATAGCGACGACTCCACCGATGATGAGGCCC
ATCCCCGGAAGCCCATCCCCACCTGGGCCCGAGGCAC
CCCGCTCAGCCAGGCTATCATTCACCAGTACTACCAG
CCACCGAACCTTCTGGAGCTCTTTGGAACCATTCTCC
CACTGGACTTGGAGGATATCTTCAAGAAGAGCAAGCC
CCGCTATCACAAGCGCACCAGCTCTGCTGTCTGGAAC
TCACCGCCCCTGCAGGGCGCCAGGGTCCCCAGCAGCC
TGGCCTACAGCCTGAAGAAGCACtga