Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Aurora C [2 - 309]

Enzyme description:- Aurora C [2 - 309]

Clone number:- DU 4009

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:- 38, 791 daltons

Purity:- >80 %

Activation protocol:-

Aurora C [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 C 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 [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, 0.1 mM sodium vanadate, 10 mM magnesium acetate

Substrate:-
RRRLSFAEPG Final concentration: 300 µM

Specific activity range:- To be determined

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Clone Data Sheet

Aurora C [2 - 309]

Protein	Aurora C [2 – 309]
Clone number	DU 4009
Species	Human
Accession number	ABO17332
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**SSPRAVVQLGKAQPAGEELAT ANQTAQQPSSPAMRRLTVDDFEIGRPLGKGKFGNVYLARLKESHFIVAL KVLFKSQIEKEGLEHQLRREIEIQAHLQHPNILRLYNYFHDARRVYLIL EYAPRGELYKELQKSEKLDEQRTATIIEELADALTYCHDKKVIHRDIKP ENLLLGFRGEVKIADFGWSVHTPSLRRKTMCGTLDYLPPEMIEGRTYDE KVDLWCIGVLCYELLVGYPPFESASHSETYRRILKVDVRFPLSMPLGAR DLISRLLRYQPLERLPLAQILKHPWVQAHSRRVPPPCAQMAS**
Native sequence	Amino acids S2 – S309 (end) of human Aurora C. Residue S29 of the fusion protein is equivalent to S2 of the native enzyme. The His(6) tag is located at residues 5 – 10. The following amino acid substitution is present: L – P, where L301 of the native enzyme is P328 of the fusion protein
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 and *Sal*1 sites of pFastBAC HTb

Complete nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGGATCCAGCTCCCCCAGAGC
TGTGGTGCAGCTGGGCAAAGCTCAACCTGCAGGCGAAGAGTTGGCTACA
GCAAACCAAACAGCCCAGCAGCCCAGCAGCCCAGCCATGCGGCGCCTCA
CAGTCGATGACTTTGAAATCGGGCGTCCCCTGGGCAAGGGGAAATTTGG
GAATGTGTACCTGGCTCGGCTCAAGGAAAGCCATTTCATTGTGGCCCTG
AAGGTTCTCTTCAAGTCGCAGATAGAGAAGGAAGGACTGGAGCACCAGC
TGCGCCGGGAAATTGAGATCCAGGCTCATCTACAACACCCCAATATCCT
GCGCCTGTATAACTATTTCCATGATGCACGCCGGGTGTACCTGATTCTG
GAATATGCTCCAAGGGGTGAGCTCTACAAGGAGCTGCAGAAAAGCGAGA
AATTAGATGAACAGCGCACAGCCACGATAATAGAGGAGTTGGCAGATGC
CCTGACCTACTGCCATGACAAGAAAGTGATTCACAGAGATATTAAGCCA
GAGAACCTGCTGCTGGGGTTCAGGGGTGAGGTGAAGATTGCAGATTTTG
GCTGGTCTGTGCACACCCCCTCCCTGAGGAGGAAGACAATGTGTGGGAC
ACTGGACTACTTGCCGCCAGAAATGATTGAGGGGAGAACATATGATGAA
AAGGTGGATTTGTGGTGCATTGGAGTGCTCTGCTATGAGCTGCTGGTGG
GATATCCACCCTTTGAGAGCGCCTCCCACAGTGAGACTTACAGACGCAT
CCTCAAGGTAGATGTGAGGTTTCCACTATCAATGCCTCTGGGGGCCCGG
GACTTGATTTCCAGGCTTCTCAGATACCAGCCCTTGGAGAGACTGCCCC
TGGCCCAGATCCTGAAGCACCCCTGGGTTCAGGCCCACTCCCGAAGGGT
GCCGCCTCCCTGTGCTCAGATGGCTTCCtga

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