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:-

Monoisotopic 38, 791.10 daltons
Average Mass 38, 815.66 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8.46

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]

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

Division of Signal Transduction Therapy

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