Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active DYRK1b [1 - 589]

Enzyme description:- DYRK1b [1 – 589]

Clone number:- DU 12362

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- Glutathione Sepharose

Calculated molecular mass:-
Monoisotopic 91, 699.89 daltons
Average Mass 91, 758.36 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8.66

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc,

Substrate:-
WOODtide [KKISGRLSPIMTEQ] Final concentration: 500 μM

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Clone Data Sheet

DYRK1b [1 - 589]

Protein	DYRK1b [1 – 589]
Clone Number	DU 12362
Species	Human
Accession number	NM_006483.1
Tags	N-terminal His(6)
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MAVPPGHGPFSGFP** **GPQEHTQVLPDVRLLPRRLPLAFRDATSAPLRKLSVDLIKTYKHINEVY** **YAKKKRRAQQAPPQDSSNKKEKKVLNHGYDDDNHDYIVRSGERWLERYE** **IDSLIGKGSFGQVVKAYDHQTQELVAIKIIKNKKAFLNQAQIELRLLEL** **MNQHDTEMKYYIVHLKRHFMFRNHLCLVFELLSYNLYDLLRNTHFRGVS** **LNLTRKLAQQLCTALLFLATPELSIIHCDLKPENILLCNPKRSAIKIVD** **FGSSCQLGQRIYQYIQSRFYRSPEVLLGTPYDLAIDMWSLGCILVEMHT** **GEPLFSGSNEVDQMNRIVEVLGIPPAAMLDQAPKARKYFERLPGGGWTL** **RRTKELRKDLVLRMLEYEPAARISPLGALQHGFFRRTADEATNTGPAGS** **SASTSPAPLDTCPSSSTASSISSSGGSSGSSSDNRTYRYSNRYCGGPGP** **PITDCEMNSPQVPPSQPLRPWAGGDVPHKTHQAPASASSLPGTGAQLPP** **QPRYLGRPPSPTSPPPPELMDVSLVGGPADCSPPHPAPAPQHPAASALR** **TRMTGGRPPLPPPDDPATLGPHLGLRGVPQSTAASS**
Native sequence	Amino acids M1 – S589 (end) of human DYRK1b. Residue M232 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Bam*H1 and *Eco*R1 site of pFastBAC GST

Nucleotide sequence of insert

ggatccATGGCCGTCCCACCGGGCCATGGTCCCTTCTCTGGCTTCCCAG
GGCCCCAGGAGCACACGCAGGTATTGCCTGATGTGCGGCTACTGCCTCG
GAGGCTGCCCCTGGCCTTCCGGGATGCAACCTCAGCCCCGCTGCGTAAG
CTCTCTGTGGACCTCATCAAGACCTACAAGCACATCAATGAGGTATACT
ATGCGAAGAAGAAGCGGCGGGCCCAGCAGGCGCCACCCCAGGATTCGAG
CAACAAGAAGGAGAAGAAGGTCCTGAACCATGGTTATGATGACGACAAC
CATGACTACATCGTGCGCAGTGGCGAGCGCTGGCTGGAGCGCTACGAAA
TTGACTCGCTCATTGGCAAAGGCTCCTTTGGCCAGGTGGTGAAAGCCTA
TGATCATCAGACCCAGGAGCTTGTGGCCATCAAGATCATCAAGAACAAA
AAGGCTTTCCTGAACCAGGCCCAGATTGAGCTGCGGCTGCTGGAGCTGA
TGAACCAGCATGACACGGAGATGAAGTACTATATAGTACACCTGAAGCG
GCACTTCATGTTCCGGAACCACCTGTGCCTGGTATTTGAGCTGCTGTCC
TACAACCTGTACGACCTCCTGCGCAACACCCACTTCCGCGGCGTCTCGC
TGAACCTGACCCGGAAGCTGGCGCAGCAGCTCTGCACGGCACTGCTCTT
TCTGGCCACGCCTGAGCTCAGCATCATTCACTGCGACCTCAAGCCCGAA
AACATCTTGCTGTGCAACCCCAAGCGCAGCGCCATCAAGATTGTGGACT
TCGGCAGCTCCTGCCAGCTTGGCCAGAGGATCTACCAGTATATCCAGAG
CCGCTTCTACCGCTCACCTGAGGTGCTCCTGGGCACACCCTACGACCTG
GCCATTGACATGTGGTCCCTGGGCTGCATCCTTGTGGAGATGCACACCG
GAGAGCCCCTCTTCAGTGGCTCCAATGAGGTCGACCAGATGAACCGCAT
TGTGGAGGTGCTGGGCATCCCACCGGCCGCCATGCTGGACCAGGCGCCC
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GAAGGACGAAAGAACTCAGGAAGGACCTGGTGCTGCGCATGCTGGAGTA
TGAGCCCGCCGCCCGCATCAGCCCCCTGGGGGCTCTGCAGCACGGCTTC
TTCCGCCGCACGGCCGACGAGGCCACCAACACGGGCCCGGCAGGCAGCA
GTGCCTCCACCTCGCCCGCGCCCCTCGACACCTGCCCCTCTTCCAGCAC
CGCCAGCTCCATCTCCAGTTCTGGAGGCTCCAGTGGCTCCTCCAGTGAC
AACCGGACCTACCGCTACAGCAACCGATATTGTGGGGGCCCTGGGCCCC
CTATCACAGACTGTGAGATGAACAGCCCCCAGGTCCCACCCTCCCAGCC
GCTGCGGCCCTGGGCAGGGGGTGATGTGCCCCACAAGACACATCAAGCC
CCTGCCTCTGCCTCGTCACTGCCTGGGACCGGGGCCCAGTTACCCCCCC
AGCCCCGATACCTTGGTCGTCCCCCATCACCAACCTCACCACCACCCCC
GGAGCTGATGGATGTGAGCCTGGTGGGCGGCCCTGCTGACTGCTCCCCA
CCTCACCCAGCGCCTGCCCCCCAGCACCCGGCTGCCTCAGCCCTCCGGA
CTCGGATGACTGGAGGTCGTCCACCCCTCCCGCCTCCTGATGACCCTGC
CACTCTGGGGCCTCACCTGGGCCTCCGTGGTGTACCCCAGAGCACAGCA
GCCAGCTCGtgagaattc