Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active DYRK2 [3 – 528]

Enzyme description:- DYRK2 [3 – 528]

Clone number:- DU 653

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 3 mg/L

Molecular mass:- 86, 533 daltons

Purity:- >75 %

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, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -20 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2−mercaptoethanol, 0.1 mM EGTA,10 mM MgAc

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

Specific activity range:- 250 – 500 U/mg

Division of Signal Tranduction Therapy

Clone Data Sheet

DYRK2 [3 – 528]

Protein	DYRK2 [3 – 528]
Clone number	DU 653
Species	Human
Accession number	NM_003583
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSRRASVGSI**DHLHVG** **SHAHGQIQVQQLFEDNSNKRTVLTTQPNGLTTVGKTGLPVVPERQLDS** **IHRRQGSSTSLKSMEGMGKVKATPMTPEQAMKQYMQKLTAFEHHEIFS** **YPEIYFLGLNAKKRQGMTGGPNNGGYDDDQGSYVQVPHDHVAYRYEVL** **KVIGKGSFGQVVKAYDHKVHQHVALKMVRNEKRFHRQAAEEIRILEHL** **RKQDKDNTMNVIHMLENFTFRNHICMTFELLSMNLYELIKKNKFQGFS** **LPLVRKFAHSILQCLDALHKNRIIHCDLKPENILLKQQGRSGIKVIDF** **GSSCYEHQRVYTYIQSRFYRAPEVILGARYGMPIDMWSLGCILAELLT** **GYPLLPGEDEGDQLACMIELLGMPSQKLLDASKRAKNFVSSKGYPRYC** **TVTTLSDGSVVLNGGRSRRGKLRGPPESREWGNALKGCDDPLFLDFLK** **QCLEWDPAVRMTPGQALRHPWLRRRLPKPPTGEKTSVKRITESTGAIT** **SISKLPPPSSSASKLRTNLAQMTDANGNIQQRTVLPKLVS**
Native sequence	Amino acids D3 – S528 (end) of human DYRK2. Residue D235 of the fusion protein is equilivalent to D3 of the native enzyme. The GST tag is located at residues 1 - 220.
Protease cleavage	Thrombin (LVPRGS) at residues 221 – 226
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX4T-1

Nucleotide sequence of insert

GGATCCATCGATCACCTGCATGTCGGCAGCCACGCTCACGGACAGATC
CAGGTTCAACAGTTGTTTGAGGATAACAGTAACAAGCGGACAGTGCTC
ACGACACAACCAAATGGGCTTACAACAGTGGGCAAAACGGGCTTGCCA
GTGGTGCCAGAGCGGCAGCTGGACAGCATTCATAGACGGCAGGGGAGC
TCCACCTCTCTAAAGTCCATGGAAGGCATGGGGAAGGTGAAAGCCACC
CCCATGACACCTGAACAAGCAATGAAGCAATACATGCAAAAACTCACA
GCCTTCGAACACCATGAGATTTTCAGCTACCCTGAAATATATTTCTTG
GGTCTAAATGCTAAGAAGCGCCAGGGCATGACAGGTGGGCCCAACAAT
GGTGGCTATGATGATGACCAGGGATCATATGTGCAGGTGCCCCACGAT
CACGTGGCTTACAGGTATGAGGTCCTCAAGGTCATTGGGAAGGGGAGC
TTTGGGCAGGTGGTCAAGGCCTACGATCACAAAGTCCACCAGCACGTG
GCCCTAAAGATGGTGCGGAATGAGAAGCGCTTCCACCGGCAAGCAGCG
GAGGAGATCCGAATCCTGGAACACCTGCGGAAGCAGGACAAGGATAAC
ACAATGAATGTCATCCATATGCTGGAGAATTTCACCTTCCGCAACCAC
ATCTGCATGACGTTTGAGCTGCTGAGCATGAACCTCTATGAGCTCATC
AAGAAGAATAAATTCCAGGGCTTCAGTCTGCCTTTGGTTCGCAAGTTT
GCCCACTCGATTCTGCAGTGCTTGGATGCTTTGCACAAAAACAGAATA
ATTCACTGTGACCTTAAGCCCGAGAACATTTTGTTAAAGCAGCAGGGT
AGAAGCGGTATTAAAGTAATTGATTTTGGCTCCAGTTGTTACGAGCAT
CAGCGTGTCTACACGTACATCCAGTCGCGTTTTTACCGGGCTCCAGAA
GTGATCCTTGGGGCCAGGTATGGCATGCCCATTGATATGTGGAGCCTG
GGCTGCATTTTAGCAGAGCTCCTGACGGGTTACCCCCTCTTGCCTGGG
GAAGATGAAGGGGACCAGCTGGCCTGTATGATTGAACTGTTGGGCATG
CCCTCACAGAAACTGCTGGATGCATCCAAACGAGCCAAAAATTTTGTG
AGCTCCAAGGGTTATCCCCGTTACTGCACTGTCACGACTCTCTCAGAT
GGCTCTGTGGTCCTAAACGGAGGCCGTTCCCGGAGGGGGAAACTGAGG
GGCCCACCGGAGAGCAGAGAGTGGGGTAACGCGCTGAAGGGGTGTGAT
GATCCCCTTTTCCTTGACTTCTTAAAACAGTGTTTAGAGTGGGATCCT
GCAGTGCGCATGACCCCAGGCCAGGCTTTGCGGCACCCCTGGCTGAGG
AGGCGGTTGCCAAAGCCTCCCACCGGGGAGAAAACGTCAGTGAAAAGG
ATAACTGAGAGCACCGGTGCTATCACATCTATATCCAAGTTACCTCCA
CCTTCTAGCTCAGCTTCCAAACTGAGGACTAATTTGGCGCAGATGACA
GATGCCAATGGGAATATTCAGCAGAGGACAGTGTTGCCAAAACTTGTT
AGCtga