Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active DYRK1a [1 – 502]

Enzyme description:- DYRK1a [1 - 502]

Clone number:- DU 19040

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 84, 458.95 daltons
Average Mass 84, 513.34 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8.68

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,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

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

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

Division of Signal Tranduction Therapy

Clone Data Sheet

DYRK1a [1 – 502]

Protein	DYRK1a [1 – 502]
Clone number	DU 19040
Species	Human
Accession number	NM_130437.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MHTGGETSA CKPSSVRLAPSFSFHAAGLQMAGQMPHSHQYSDRRQPNISDQQVSALS YSDQIQQPLTNQVMPDIVMLQRRMPQTFRDPATAPLRKLSVDLIKTYK HINEVYYAKKKRRHQQGQGDDSSHKKERKVYNDGYDDDNYDYIVKNGE KWMDRYEIDSLIGKGSFGQVVKAYDRVEQEWVAIKIIKNKKAFLNQAQ IEVRLLELMNKHDTEMKYYIVHLKRHFMFRNHLCLVFEMLSYNLYDLL RNTNFRGVSLNLTRKFAQQMCTALLFLATPELSIIHCDLKPENILLCN PKRSAIKIVDFGSSCQLGQRIYQYIQSRFYRSPEVLLGMPYDLAIDMW SLGCILVEMHTGEPLFSGANEVDQMNKIVEVLGIPPAHILDQAPKARK FFEKLPDGTWNLKKTKDGKREYKPPGTRKLHNILGVETGGPGGRRAGE SGHTVADYLKFKDLILRMLDYDPKTRIQPYYALQHSFFKKTADEGTNT SNSVSTSPAMEQS**
Native sequence	Amino acids M1 – S502 of human DYRK1a. [Full length protein ends at residue V7540] Residue M232 of the fusion protein is equilivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1site of pGEX6P-1

Nucleotide sequence of insert

ggatccATGCATACAGGAGGAGAGACTTCAGCATGCAAACCTTCATCT
GTCCGGCTTGCACCGTCGTTCTCATTCCATGCTGCTGGCCTTCAGATG
GCTGGACAGATGCCCCACTCACACCAGTACAGTGACCGTCGCCAGCCG
AACATAAGTGACCAGCAGGTGTCTGCCTTATCATATTCTGACCAGATT
CAGCAACCTCTAACTAACCAGGTGATGCCTGATATTGTCATGTTACAG
AGGCGGATGCCCCAAACCTTCCGTGATCCAGCAACTGCTCCTCTGAGA
AAACTCTCTGTGGACTTGATCAAAACATACAAGCATATTAATGAGGTT
TACTATGCAAAAAAGAAGCGAAGACACCAACAGGGCCAGGGGGACGAT
TCCAGTCATAAGAAGGAGCGGAAGGTTTACAATGATGGTTACGATGAT
GATAACTATGATTATATTGTAAAAAACGGGGAAAAGTGGATGGATCGG
TATGAAATCGACTCCTTAATAGGCAAAGGTTCATTTGGACAGGTTGTG
AAAGCTTATGACAGAGTGGAGCAAGAATGGGTCGCCATTAAAATCATC
AAGAACAAGAAAGCGTTTCTGAATCAAGCCCAGATAGAAGTGCGGCTG
CTTGAGCTCATGAACAAACACGACACTGAAATGAAGTACTACATAGTG
CATTTGAAACGCCACTTTATGTTTCGAAACCATCTCTGTTTAGTGTTT
GAAATGCTGTCCTATAACCTCTATGATTTGTTGAGAAACACCAACTTC
CGAGGGGTCTCTTTGAACCTAACACGAAAGTTTGCGCAACAGATGTGC
ACAGCATTGCTTTTTCTTGCGACTCCAGAACTTAGTATCATTCACTGT
GACTTAAAGCCTGAAAATATCCTTCTTTGTAACCCCAAACGCAGTGCA
ATCAAGATAGTTGACTTTGGCAGTTCTTGTCAGTTGGGGCAGAGGATA
TACCAGTATATTCAGAGTCGCTTTTATCGGTCTCCAGAGGTGCTACTG
GGAATGCCTTATGACCTTGCCATTGATATGTGGTCCCTCGGGTGTATT
TTGGTTGAAATGCACACTGGAGAACCTCTGTTCAGTGGTGCCAATGAG
GTAGATCAGATGAATAAAATAGTGGAAGTTCTGGGTATTCCACCTGCT
CATATTCTTGACCAAGCACCAAAAGCAAGAAAGTTCTTTGAGAAGTTG
CCAGATGGCACTTGGAACTTAAAGAAGACCAAAGATGGAAAACGGGAG
TACAAACCACCAGGAACCCGTAAACTTCATAACATTCTTGGAGTGGAA
ACAGGAGGACCTGGTGGGCGACGTGCTGGGGAGTCAGGTCATACGGTC
GCTGACTACTTGAAGTTCAAAGACCTCATTTTAAGGATGCTTGATTAT
GACCCCAAAACTCGAATTCAACCTTATTATGCTCTGCAGCACAGTTTC
TTCAAGAAAACAGCTGATGAAGGTACAAATACAAGTAATAGTGTATCT
ACAAGCCCCGCCATGGAGCAGTCTtaagcggccgc