Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active DYRK1a [1 – 499]

Enzyme description:- DYRK1a [1 - 499]

Clone number:- DU 1501

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- >2 mg/L

Calculated molecular mass:- 85, 087 daltons

Purity:- >70 %

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:- 150 – 300 U/mg

Division of Signal Tranduction Therapy

Clone Data Sheet

DYRK1a [1 – 499]

Protein	DYRK1a [1 – 499]
Clone number	DU 1501
Species	Rat
Accession number	X79769
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKW RNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNML GGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFL SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLY MDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKSDLVPRGSRRASVGSPGIQT**MHTGGETS** **ACKPSSVRLAPSFSFHAAGLQMAAQMPHSHQYSDRRQPNIS** **DQQVSALSYSDQIQQPLTNQVMPDIVMLQRRMPQTFRDPAT** **APLRKLSVDLIKTYKHINEVYYAKKKRRHQQGQGDDSSHKK** **ERKVYNDGYDDDNYDYIVKNGEKWMDRYEIDSLIGKGSFGQ** **VVKAYDRVEQEWVAIKIIKNKKAFLNQAQIEVRLLELMNKH** **DTEMKYYIVHLKRHFMFRNHLCLVFEMLSYNLYDLLRNTNF** **RGVSLNLTRKFAQQMCTALLFLATPELSIIHCDLKPENILL** **CNPKRSAIKIVDFGSSCQLGQRIYQYIQSRFYRSPEVLLGM** **PYDLAIDMWSLGCILVEMHTGEPLFSGANEVDQMNKIVEVL** **GIPPAHILDQAPKARKFFEKLPDGTWSLKKTKDGKREYKPP** **GTRKLHNILGVETGGPGGRRAGESGHTVADYLKFKDLILRM** **LDYDPKTRIQPYYALQHSFFKKTADEGTNTSNSVSTSPAMH** **GL**
Native sequence	Amino acids M1 – L499 of rat DYRK1a. [Full length protein ends at residue S763] Residue M239 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	Thrombin (LVPRGS) at residues 221 – 226

Cloning sites

*Bam*H1 site of pGEX-4T1

Nucleotide sequence of insert

GGATCCCCGGGAATTCAGACGATGCATACAGGAGGAGAGACTTCAGCA
TGCAAACCTTCATCTGTCCGGCTCGCACCGTCATTCTCATTCCATGCT
GCTGGCCTTCAGATGGCTGCACAGATGCCCCATTCACATCAGTACAGT
GACCGTCGCCAGCCAAACATAAGTGACCAGCAGGTTTCTGCCTTATCA
TATTCTGACCAGATTCAGCAACCTCTAACTAACCAGGTGATGCCTGAC
ATTGTCATGTTACAGAGGCGGATGCCCCAAACCTTCCGTGATCCAGCA
ACTGCTCCTCTGAGAAAACTCTCTGTGGACTTGATCAAAACATACAAG
CATATTAATGAGGTTTACTATGCAAAAAAGAAGCGAAGACACCAACAG
GGCCAGGGGGACGATTCTAGTCATAAGAAGGAGCGGAAGGTTTACAAC
GATGGTTACGATGACGATAACTATGATTACATTGTAAAAAACGGAGAA
AAGTGGATGGACCGTTATGAAATCGACTCCTTAATAGGCAAAGGTTCA
TTTGGACAGGTTGTGAAAGCATATGACAGAGTGGAACAAGAATGGGTC
GCCATTAAAATCATCAAGAACAAGAAGGCGTTTCTGAATCAAGCGCAG
ATAGAAGTGCGGCTGCTTGAGCTCATGAACAAACATGACACTGAGATG
AAGTACTACATAGTGCATTTGAAACGCCACTTTATGTTTCGGAACCAT
CTCTGTCTAGTGTTTGAAATGCTGTCCTATAATCTCTATGATTTGTTG
AGGAACACCAACTTCCGAGGGGTCTCTTTGAACCTGACACGAAAGTTT
GCACAACAGATGTGCACAGCATTGCTTTTCCTTGCGACTCCAGAACTT
AGTATCATTCACTGTGACTTAAAGCCGGAGAACATCCTTCTGTGTAAC
CCCAAACGAAGTGCAATCAAGATTGTTGACTTTGGCAGCTCTTGTCAG
TTGGGGCAGAGGATATACCAGTATATTCAGAGTCGCTTCTATCGGTCT
CCAGAGGTGCTACTGGGAATGCCTTATGACCTTGCTATCGACATGTGG
TCCCTTGGATGTATCTTGGTTGAAATGCACACTGGAGAGCCTCTGTTC
AGTGGTGCCAATGAGGTGGATCAGATGAATAAAATAGTGGAAGTCTTG
GGCATCCCACCTGCTCATATTCTTGACCAAGCACCGAAAGCAAGAAAG
TTCTTTGAGAAGTTGCCTGATGGCACTTGGAGCTTAAAGAAGACCAAA
GATGGAAAACGGGAGTACAAACCACCAGGAACCCGTAAACTTCATAAT
ATTCTTGGAGTGGAAACAGGAGGACCTGGCGGGCGGCGTGCTGGGGAG
TCAGGTCACACTGTAGCTGACTACTTGAAGTTCAAAGACCTCATTTTA
AGGATGCTTGATTATGACCCCAAAACTCGGATTCAACCTTATTATGCC
CTGCAGCACAGTTTTTTCAAGAAAACAGCTGATGAAGGTACAAACACA
AGCAACAGTGTGTCCACCAGCCCTGCCATGCATGGACTGtgagacaca
tagtccccaggtgcgccagcagtttccggctcc