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

Expression level:- >2 mg/L

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:- >70 %

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:- 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:- To be determined

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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
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CGAGGGGTCTCTTTGAACCTAACACGAAAGTTTGCGCAACAGATGTGC
ACAGCATTGCTTTTTCTTGCGACTCCAGAACTTAGTATCATTCACTGT
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TACCAGTATATTCAGAGTCGCTTTTATCGGTCTCCAGAGGTGCTACTG
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ACAGGAGGACCTGGTGGGCGACGTGCTGGGGAGTCAGGTCATACGGTC
GCTGACTACTTGAAGTTCAAAGACCTCATTTTAAGGATGCTTGATTAT
GACCCCAAAACTCGAATTCAACCTTATTATGCTCTGCAGCACAGTTTC
TTCAAGAAAACAGCTGATGAAGGTACAAATACAAGTAATAGTGTATCT
ACAAGCCCCGCCATGGAGCAGTCTtaagcggccgc