Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active DYRK3 [1 – 588]

Enzyme description:- DYRK3 [1 – 588]

Clone number:- DU 3371

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 1 mg/L

Calculated molecular mass:-
Monoisotopic 92, 478.50 daltons
Average Mass 92, 537.90 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 9.02

Purity:- >75 %

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

DYRK3 [1 – 588]

Protein	DYRK3 [1 – 588]
Clone number	DU 3371
Species	Human
Accession number	AY590695
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MGGTARGPG** **RKDAGPPGAGLPPQQRRLGDGVYDTFMMIDETKCPPCSNVLCNPSEPP** **PPRRLNMTTEQFTGDHTQHFLDGGEMKVEQLFQEFGNRKSNTIQSDGI** **SDSEKCSPTVSQGKSSDCLNTVKSNSSSKAPKVVPLTPEQALKQYKHH** **LTAYEKLEIINYPEIYFVGPNAKKRHGVIGGPNNGGYDDADGAYIHVP** **RDHLAYRYEVLKIIGKGSFGQVARVYDHKLRQYVALKMVRNEKRFHRQ** **AAEEIRILEHLKKQDKTGSMNVIHMLESFTFRNHVCMAFELLSIDLYE** **LIKKNKFQGFSVQLVRKFAQSILQSLDALHKNKIIHCDLKPENILLKH** **HGRSSTKVIDFGSSCFEYQKLYTYIQSRFYRAPEIILGSRYSTPIDIW** **SFGCILAELLTGQPLFPGEDEGDQLACMMELLGMPPPKLLEQSKRAKY** **FINSKGIPRYCSVTTQADGRVVLVGGRSRRGKKRGPPGSKDWGTALKG** **CDDYLFIEFLKRCLHWDPSARLTPAQALRHPWISKSVPRPLTTIDKVS** **GKRVVNPASAFQGLGSKLPPVVGIANKLKANLMSETNGSIPLCSVLPK** **LIS**
Native sequence	Amino acids M1 – S588 (end) of human DYRK3. Residue M232 of the fusion protein is equilivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 and *Not*1 site of pGEX6P-1

Nucleotide sequence of insert

ggatccATGGGAGGCACAGCTCGTGGGCCTGGGCGGAAGGATGCGGGG
CCGCCTGGGGCCGGGCTCCCGCCCCAGCAGCGGAGGTTGGGGGATGGT
GTCTATGACACCTTCATGATGATAGATGAAACCAAATGTCCCCCCTGT
TCAAATGTACTCTGCAATCCTTCTGAACCACCTCCACCCAGAAGACTA
AATATGACCACTGAGCAGTTTACAGGAGATCATACTCAGCACTTTTTG
GATGGAGGTGAGATGAAGGTAGAACAGCTGTTTCAAGAATTTGGCAAC
AGAAAATCCAATACTATTCAGTCAGATGGCATCAGTGACTCTGAAAAA
TGCTCTCCTACTGTTTCTCAGGGTAAAAGTTCAGATTGCTTGAATACA
GTAAAATCCAACAGTTCATCCAAGGCACCCAAAGTGGTGCCTCTGACT
CCAGAACAAGCCCTGAAGCAATATAAACACCACCTCACTGCCTATGAG
AAACTGGAAATAATTAATTATCCAGAAATTTACTTTGTAGGTCCAAAT
GCCAAGAAAAGACATGGAGTTATTGGTGGTCCCAATAATGGAGGGTAT
GATGATGCAGATGGGGCCTATATTCATGTACCTCGAGACCATCTAGCT
TATCGATATGAGGTGCTGAAAATTATTGGCAAGGGGAGTTTTGGGCAG
GTGGCCAGGGTCTATGATCACAAACTTCGACAGTACGTGGCCCTAAAA
ATGGTGCGCAATGAGAAGCGCTTTCATCGTCAAGCAGCTGAGGAGATC
CGGATTTTGGAGCATCTTAAGAAACAGGATAAAACTGGTAGTATGAAC
GTTATCCACATGCTGGAAAGTTTCACATTCCGGAACCATGTTTGCATG
GCCTTTGAATTGCTGAGCATAGACCTTTATGAGCTGATTAAAAAAAAT
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CTTGCAGAACTTTTAACAGGACAGCCTCTCTTCCCTGGAGAGGATGAA
GGAGACCAGTTGGCCTGCATGATGGAGCTTCTAGGGATGCCACCACCA
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GTGCTTGTGGGGGGTCGCTCACGTAGGGGTAAAAAGCGGGGTCCCCCA
GGCAGCAAAGACTGGGGGACAGCACTGAAAGGGTGTGATGACTACTTG
TTTATAGAGTTCTTGAAAAGGTGTCTTCACTGGGACCCCTCTGCCCGC
TTGACCCCAGCTCAAGCATTAAGACACCCTTGGATTAGCAAGTCTGTC
CCCAGACCTCTCACCACCATAGACAAGGTGTCAGGGAAACGGGTAGTT
AATCCTGCAAGTGCTTTCCAGGGATTGGGTTCTAAGCTGCCTCCAGTT
GTTGGAATAGCCAATAAGCTTAAAGCTAACTTAATGTCAGAAACCAAT
GGTAGTATACCCCTATGCAGTGTATTGCCAAAACTGATTAGCtaggcg
gccgc