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## Standard Operating Procedure

### Preparation of active DYRK2 [3 – 528]

<b><u>Enzyme description:-</u></b>	DYRK2 [3 – 528]
<b><u>Clone number:-</u></b>	DU 653
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	<i>E.coli</i>
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH Sepharose
<b><u>Expression level:-</u></b>	3 mg/L
<b><u>Molecular mass:-</u></b>	86, 533 daltons
<b><u>Purity:-</u></b>	>75 %
<b><u>Activation protocol:-</u></b>	Constitutively active
<b><u>Enzyme storage buffer:-</u></b>	
	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
<b><u>Storage temperature:-</u></b>	-20 °C
<b><u>Assay:-</u></b>	Standard filter binding assay
<b><u>Assay buffer:-</u></b>	
	50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc
<b><u>Substrate:-</u></b>	
WOODtide [KKISGRLSPIMTEQ]	Final concentration: 500 $\mu$ M
<b><u>Specific activity range:-</u></b>	250 – 500 U/mg

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## 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**

MSPILGYWKIKGLVQPTRLLEYLEEKYEHLERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKL TQSM IIRY IADKHNMLGGCPKERA EISM L E  
GAVLDIRYGVSRIAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN  
GDHVTHPDFM LYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY  
LKSSKYIAWPLQGWQATFGGGDHPKSDLVPRGSRRASVGS **IDHLHVG**  
**SHAHGQIQVQQLFEDNSNKRTVLT TQPNGLTTVGKTGLPVVPERQLDS**  
**IHRROGSSSTLKSMEGMGKVKATPMTPEQAMKQYMOKLTAFEHHEIFS**  
**YPEIYFLGLNAKKRQGMTGGPNNGGYDDDQGSYVQVPHDHVAYRYEVL**  
**KVIGKGSFGQVVKAYDHKVHQHVALKMVRNEKRFHRQAAEEIRILEHL**  
**RKQDKDNTMNVIHMLENFTFRNHICMTFELLSMNLIELIKKNKFQGS**  
**LPLVRKFAHSILQCLDALHKNRIHCDLKPENILLKQQGRSGIKVIDF**  
**GSSCYEHQRVYTYIQSRFYRAPEVILGARYGMPIDMWSLGCILAELLT**  
**GYPLLPGEDEGDQLACMIELLGMP SQKLLDASKRAKNFVSSKGYPRYC**  
**TVTTLSDGSVVLNNGRSRRGKLRGPPESREWGNALKGCD DPLFLDFLK**  
**QCLEWDP AVRMPGQALRHPWLRRLPKPPTGEKTSVKRITESTGAIT**  
**SISKLPPPSSSASKLRTNLAQMTDANGNIQORTVLPKLV S**

**Native sequence** Amino acids D3 – S528 (end) of human DYRK2.  
Residue D235 of the fusion protein is equivalent 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

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## Nucleotide sequence of insert

GGATCCATCGATCACCTGCATGTCGGCAGCCACGCTCACGGACAGATC  
CAGGTTCAACAGTTGTTTGAGGATAACAGTAACAAGCGGACAGTGCTC  
ACGACACAACCAAATGGGCTTACAACAGTGGGCAAAACGGGCTTGCCA  
GTGGTGCCAGAGCGGCAGCTGGACAGCATTTCATAGACGGCAGGGGAGC  
TCCACCTCTCTAAAGTCCATGGAAGGCATGGGGAAGGTGAAAGCCACC  
CCCATGACACCTGAACAAGCAATGAAGCAATACATGCAAAAACTCACA  
GCCTTCGAACACCATGAGATTTTCAGCTACCCTGAAATATATTTCTTG  
GGTCTAAATGCTAAGAAGCGCCAGGGCATGACAGGTGGGCCCAACAAT  
GGTGGCTATGATGATGACCAGGGATCATATGTGCAGGTGCCCCACGAT  
CACGTGGCTTACAGGTATGAGGTCCTCAAGGTCATTGGGAAGGGGAGC  
TTTGGGCAGGTGGTCAAGGCCTACGATCACAAAGTCCACCAGCACGTG  
GCCCTAAAGATGGTGC GGAATGAGAAGCGCTTCCACCGCAAGCAGCG  
GAGGAGATCCGAATCCTGGAACACCTGCGGAAGCAGGACAAGGATAAC  
ACAATGAATGTTCATCCATATGCTGGAGAATTTACCTTCCGCAACCAC  
ATCTGCATGACGTTTGAGCTGCTGAGCATGAACCTCTATGAGCTCATC  
AAGAAGAATAAATTCAGGGCTTCAGTCTGCCTTTGGTTTCGCAAGTTT  
GCCCACTCGATTCTGCAGTGCTTGGATGCTTTGCACAAAAACAGAATA  
ATTCACTGTGACCTTAAGCCCGAGAACATTTTGTAAAGCAGCAGGGT  
AGAAGCGGTATTAAAGTAATTGATTTTGGCTCCAGTTGTTACGAGCAT  
CAGCGTGTCTACACGTACATCCAGTCGCGTTTTTACC GGGCTCCAGAA  
GTGATCCTTGGGGCCAGGTATGGCATGCCATTGATATGTGGAGCCTG  
GGCTGCATTTTAGCAGAGCTCCTGACGGGTTACCCCTCTTGCTGGG  
GAAGATGAAGGGGACCAGCTGGCCTGTATGATTGAACTGTTGGGCATG  
CCCTCACAGAAACTGCTGGATGCATCCAAACGAGCCAAAAATTTGTG  
AGCTCCAAGGGTTATCCCGTTACTGCACTGTCACGACTCTCTCAGAT  
GGCTCTGTGGTCCTAAACGGAGGCCGTTCCCGGAGGGGAAACTGAGG  
GGCCACCGGAGAGCAGAGAGTGGGGTAACGCGCTGAAGGGGTGTGAT  
GATCCCTTTTCTTGACTTCTTAAACAGTGTTTAGAGTGGGATCCT  
GCAGTGCGCATGACCCAGGCCAGGCTTTGCGGCACCCCTGGCTGAGG  
AGGCGGTTGCCAAAGCCTCCACCGGGGAGAAAACGTCAGTGAAAAGG  
ATAACTGAGAGCACCGGTGCTATCACATCTATATCCAAGTTACCTCCA  
CCTTCTAGCTCAGCTTCCAAACTGAGGACTAATTTGGCGCAGATGACA  
GATGCCAATGGGAATATTCAGCAGAGGACAGTGTTGCCAAAACTTGTT  
AGCtga