

# University of Dundee

## 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  $\mu$ M

**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** MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE  
GAVLDIRYGVSR IAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN  
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY  
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGPLGSMGGTARGPG  
**RKDAGPPGAGLPPQORRLGDGVYDTFMMIDETKPPCSNVLCNPSEPP**  
**PPRRLNMTTEQFTGDHTQHFLDGGEMKVEQLFQEFGNRKSNTIQSDGI**  
**SDSEKCSPTVSQKSSDCLNTVKSNSSSKAPKVVPLTPEQALKQYKHH**  
**LTAYEKLEIINYPEIYFVGNAPKRRHGVIGGPNNGGYDDADGAYIHVP**  
**RDHLAYRYEVLKIIGKGSFGQVARVYDHKLRQYVALKMVRNEKRFHRQ**  
**AAEEIRILEHLKKQDKTGSMNVIHMLESFTFRNHVCMAFELLSIDLIE**  
**LIKKNKFQGSVQLVRKFAQSILQSLDALHKNKI IHCDLKPENILLKH**  
**HGRSSTKVIDFGSSCFEYQKLYTYIQSRFYRAPEIILGSRYSTPIDIW**  
**SFGCILAE LLTGQPLFPGEDEGDQLACMELLGMPPP KLEQSKRAKY**  
**FINSKGIPRYCSVTTQADGRVVLVGGRSRRGKKRGGPPGSKDWGTALKG**  
**CDDYLFIEFLKRCLHWDPSARLTPAQALRHPWISKSVPRPLTTIDKVS**  
**GKRVVNPASAFQGLGSKLPPVVGIANKLANLMSETNGSIPLCSVLPK**  
**LIS**

**Native sequence** Amino acids M1 – S588 (end) of human DYRK3.  
Residue M232 of the fusion protein is equivalent 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

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## Nucleotide sequence of insert

ggatccATGGGAGGCACAGCTCGTGGGCCTGGGCGGAAGGATGCGGGG  
CCGCCTGGGGCCGGGCTCCCCCCCCAGCAGCGGAGGTTGGGGGATGGT  
GTCTATGACACCTTCATGATGATAGATGAAACCAAATGTCCCCCTGT  
TCAAATGTACTCTGCAATCCTTCTGAACCACCTCCACCCAGAAGACTA  
AATATGACCACTGAGCAGTTTACAGGAGATCATACTCAGCACTTTTTG  
GATGGAGGTGAGATGAAGGTAGAACAGCTGTTTCAAGAATTTGGCAAC  
AGAAAATCCAATACTATTTCAGTCAGATGGCATCAGTGACTCTGAAAAA  
TGCTCTCCTACTGTTTCTCAGGGTAAAAGTTCAGATTGCTTGAATACA  
GTAAAATCCAACAGTTCATCCAAGGCACCCAAAGTGGTGCCTCTGACT  
CCAGAACAAGCCCTGAAGCAATATAAACACCACCTCACTGCCTATGAG  
AAACTGGAAATAATTAATTATCCAGAAATTTACTTTGTAGGTCCAAAT  
GCCAAGAAAAGACATGGAGTTATTGGTGGTCCCAATAATGGAGGGTAT  
GATGATGCAGATGGGGCCTATATTCATGTACCTCGAGACCATCTAGCT  
TATCGATATGAGGTGCTGAAAATTTATTGGCAAGGGGAGTTTTTGGGCAG  
GTGGCCAGGGTCTATGATCACAACCTTCGACAGTACGTGGCCCTAAAA  
ATGGTGCGAATGAGAAGCGCTTTCATCGTCAAGCAGCTGAGGAGATC  
CGGATTTTGGAGCATCTTAAGAAACAGGATAAAAAGTGGTAGTATGAAC  
GTTATCCACATGCTGGAAAGTTTCACATTCGGAACCATGTTTGCATG  
GCCTTTGAATTGCTGAGCATAGACCTTTATGAGCTGATTAATAAAAAAT  
AAGTTTCAGGGTTTTAGCGTCCAGTTGGTACGCAAGTTTGCCAGTCC  
ATCTTGCAATCTTTGGATGCCCTCCACAAAAATAAGATTATTCAGTGC  
GATCTGAAGCCAGAAAACATTCTCCTGAAACACCACGGGCGCAGTTCA  
ACCAAGGTCATTGACTTTGGGTCCAGCTGTTTCGAGTACCAGAAGCTC  
TACACATATATCCAGTCTCGGTTCTACAGAGCTCCAGAAATCATCTTA  
GGAAGCCGCTACAGCACACCAATTGACATATGGAGTTTTTGGCTGCATC  
CTTGCAGAACTTTTAAACAGGACAGCCTCTCTTCCCTGGAGAGGATGAA  
GGAGACCAGTTGGCCTGCATGATGGAGCTTCTAGGGATGCCACCACCA  
AACTTCTGGAGCAATCCAACGTGCCAAGTACTTTATTAATTCCAAG  
GGCATAACCCGCTACTGCTCTGTGACTACCCAGGCAGATGGGAGGGTT  
GTGCTTGTGGGGGGTTCGCTCACGTAGGGGTAAAAGCGGGGTCCCCCA  
GGCAGCAAAGACTGGGGGACAGCACTGAAAGGGTGTGATGACTACTTG  
TTTATAGAGTTCTTGAAAAGGTGTCTTCACTGGGACCCCTCTGCCCCG  
TTGACCCAGCTCAAGCATTAAAGACACCCTTGGATTAGCAAGTCTGTC  
CCCAGACCTCTCACCACCATAGACAAGGTGTCAGGGAAACGGGTAGTT  
AATCCTGCAAGTGCTTTCCAGGGATTGGGTTCTAAGCTGCCTCCAGTT  
GTTGGAATAGCCAATAAGCTTAAAGCTAACTTAATGTCAGAAACCAAT  
GGTAGTATACCCCTATGCAGTGTATTGCCAAAAGTATTAGCTagggc  
gccgc