

Certificate of Analysis

DYRK3, active **(Recombinant enzyme expressed in Sf21 insect cells)** **Item # 15-002, 15-002-K, 15-002M** **Parent Lot # D15MP017N**

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal GST-tagged and C-terminal 6His tagged, recombinant, human DYRK3 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 94 kDa.

Specific Activity (Parent lot# D15MP017N): 1047 U/mg, where one unit of DYRK3 activity is defined as 1 nmol phosphate incorporated into 250 μ M RRRFRPASPLRGPPK per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.52 mg/ml of enzyme in 50 mM Tris/HCl pH7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

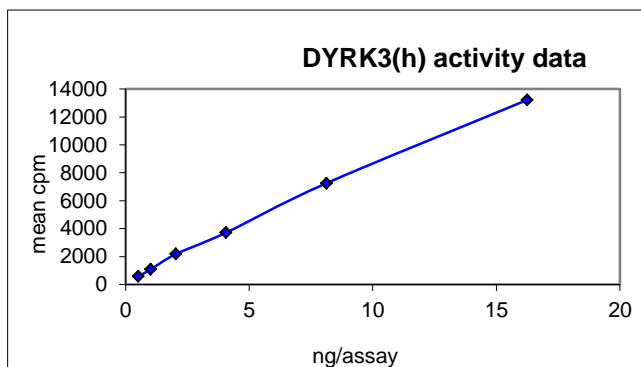
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.5–16.3 ng of this lot of enzyme phosphorylated 250 μ M RRRFRPASPLRGPPK in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as DYRK3 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH 7.0, 1 mM EDTA.
- 2. RRRFRPASPLRGPPK:** Use at a final assay concentration of 250 μ M. Prepare a 2.5 mM stock and add 2.5 μ l of stock per assay point.
- 3. DYRK3, active:** Dilute with 20 mM MOPS/NaOH pH7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 0.5–16.3 ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of RRRFRPASPLRGPPK.
3. Add **2.5 μ l (0.5–16.3 ng) DYRK3, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the dried filtermat to a sealable plastic bag and add 4 ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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DYRK3, active Sequence Information

<u>Protein</u>	Human DYRK3
<u>Tags</u>	N-terminal GST and C-terminal 6His
<u>Native sequence</u>	M230 of the recombinant protein is equivalent to M1 of human DYRK3
<u>Accession number</u>	GenBank NM_003582.2

Recombinant DYRK3 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPQID  KYLKSSKYIA  WPLQGQWQATF  GGGDHPPKSD  LVPRGSKEFEM  GGTARGPGRK
241  DAGPPGAGLP  PQQRRLGDGV  YDTFMMIDET  KCPPCSNVLC  NPSEPPPPRR  LNMTTEQFTG
301  DHTQHFLDGG  EMKVEQLFQE  FGNRKSNTIQ  SDGISDSEKC  SPTVSQ GKSS  DCLNTVKSNS
361  SSKAPKVVPL  TPEQALKQYK  HHLTAYEKLE  IINYPEIYFV  GPNAKRRHGV  IGGPNNGGYD
421  DADGAYIHVP  RDHLAYRYEV  LKIIGKGSFG  QVARVYDHKL  RQYVALKMVR  NEKRFRQAAA
481  EEIRILEHLK  KQDKTGS MNV  IHMLESFTFR  NHVCMAFELL  SIDLYELIKK  NKFQGF SVQL
541  VRKFAQSILQ  SLDALHKNKI  IHCDLKPENI  LLKHHGRSST  KVIDFGSSCF  EYQKLYTYIQ
601  SRFYRAPEII  LGSRYSTPID  IWSFGCILAE  LLTGQPLFPG  EDEGDQLACM  MELLGMPPPK
661  LLEQSKRAKY  FINSKGI PRY  CSVTTQADGR  VVLVGGRSRR  GKRRGPPGSK  DWGTALKGCD
721  DYLFIEFLKR  CLHWDPSARL  TPAQALRHPW  ISKSVRPLT  TIDKVS GKRV  VNPASAFQGL
781  GSKLPPVVG I  ANKLANLMS  ETNGSIPLCS  VLPKLISGPG  PGHHHHHH

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Recombinant DYRK3 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatatt  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttctcta  ttatattgat
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361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
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1201  ggtccaaatg  ccaagaaaag  acatggagtt  attggtggtc  ccaataatgg  agggatgat
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2401 gaaaccaatg gtagtatacc cctatgcagt gtattgcaa aactgattag cggcccgggc
2461 cctggccatc accatcacca tcactaa
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