

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 = 94kDa.

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

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

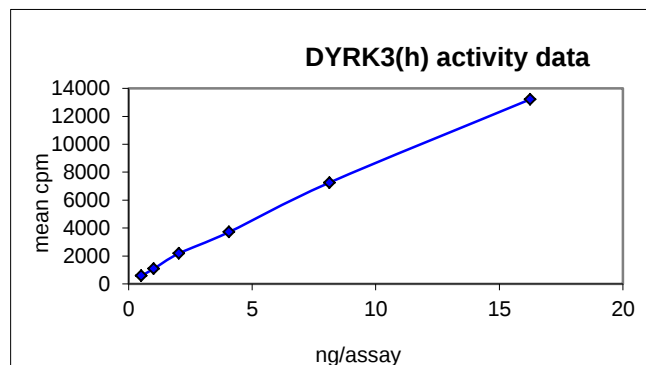
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

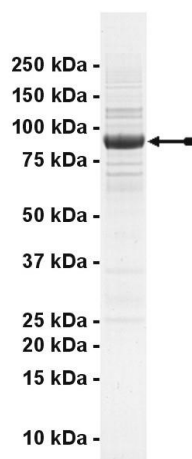
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.51–16.25ng of this lot of enzyme phosphorylated 250µM 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.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of DYRK3, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. RRRFRPASPLRGPPK:** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
- 3. DYRK3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.51–16.25ng per assay point.
- 4. [γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/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 (0.51–16.25ng) 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 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the dried filtermat to a sealable plastic bag and add 4ml 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  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSKEFM  GGTARGPGRK
241 DAGPPGAGLP  PQRRRLGDGV  YDTFMMIDET  KCPPCSNVLC  NPSEPPPPRR  LNMTEQFTG
301 DHTQHFLDGG  EMKVEQLFQE  FGNRKSNTIQ  SDGISDSEKC  SPTVSQ GKSS  DCLNTVKSNS
361 SSKAPKVVPL  TPEQALKQYK  HHLTAYEKE  IINYPEIYFV  GPNAKKRHGV  IGGPNNGGYD
421 DADGAYIHVP  RDHLAYRYEV  LKIIGKGSFG  QVARVYDHKL  RQYVALKMVR  NEKRFHRQAA
481 EEIRILEHLK  KQDKTGSMNV  IHMLESFTFR  NHVCMAFELL  SIDLYELIKK  NKFQGFVQL
541 VRKFAQSILQ  SLDALHKNKI  IHCDLKPENI  LLKHHGRSST  KVIDFGSSCF  EYQKLYTYIQ
601 SRFYRAPEII  LGSRYSTPID  IWSFGCILAE  LLTGQPLFPG  EDEGDQLACM  MELLGMPPPK
661 LLEQSKRAKY  FINSKGIPRY  CSVTQTADGR  VVLVGGRSRR  GKKRGPPGSK  DWGTALKGCD
721 DYLFIEFLKR  CLHWDPSARL  TPAQALRHPW  ISKSVPRPLT  TIDKVS GKRV  VNPASAFQGL
781 GSKLPPVVG  ANKLKANLMS  ETNGSIPLCS  VLPKLISGPG  PGHHHHHH

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Recombinant DYRK3 nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
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121 tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcccta  ttatatgat
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241 atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcgggtttg
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361 gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcggtt  atgtcataaa
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1261 gatgcagatg  gggcctatat  tcatgtacct  cgagaccatc  tagcttatcg  atatgaggtg
1321 ctgaaaatta  ttggcaaggg  gagttttggg  caggtggcca  gggctctatga  tcacaaactt

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1381 cgacagtacg tggccctaaa aatggtgcgc aatgagaagc gctttcatcg tcaagcagct
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1501 atccacatgc tggaaagttt cacattccgg aaccatgttt gcatggcctt tgaattgctg
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2401 gaaaccaatg gtagtatacc cctatgcagt gtattgcaa aactgattag cggcccgggc
2461 cctggccatc accatcacca tcactaa

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