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Certificate of Analysis

DYRK1B, active
(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-944, 14-944-K, 14-944M

Parent Lot # D14BP004N

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, recombinant, human DYRK1B full length, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose followed by gel filtration.
Purity 90% by SDS-PAGE and Coomassie blue staining. MW = 96kDa.

Specific Activity (Parent lot# D14BP004N): 2695U/mg, where one unit of DYRK1B, active activity is defined as 1nmol phosphate incorporated into 50µM (RRRFRPASPLRGPPK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.523mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 20mM β-glycerophosphate, 10mM NaF, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

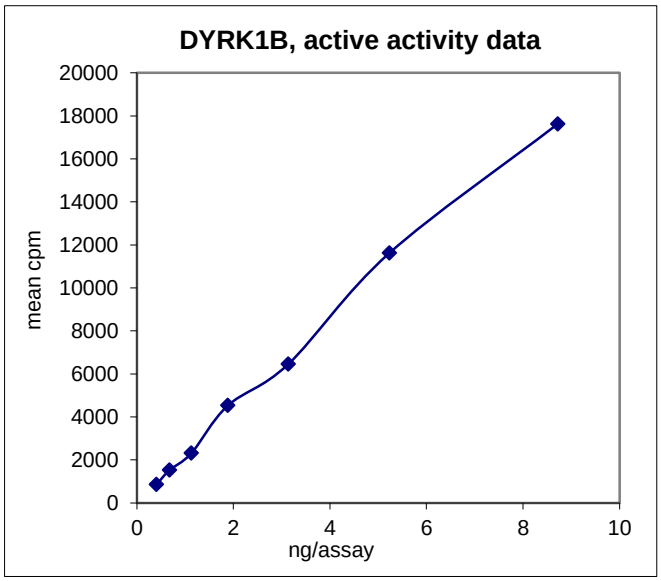
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

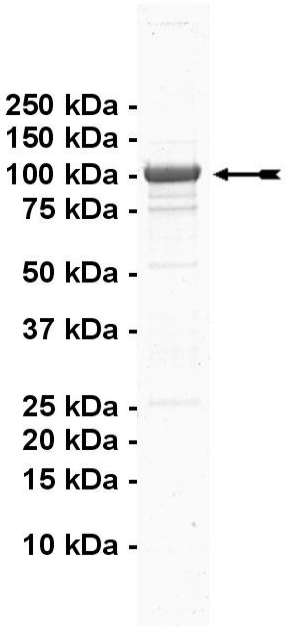
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.4–8.7ng of this lot of enzyme phosphorylated 50µM (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 DYRK1B 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 DYRK1B, 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 50µM. Prepare a 2.5mM stock and add 0.5µl of stock per assay point.
3. **BSA**: Use at a final assay concentration of 0.5%(w/v). Prepare a 10%(w/v) solution in water and use 1.25µl per well.
4. **DYRK1B, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.4–8.7ng per assay point.
5. **[γ-^{33}P]ATP:** 2.5 x MgAc/[γ^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 1.25µl 10% BSA to wells
3. Add 0.5µl of (RRRFRPASPLRGPPK).
4. Add **2.5µl (0.4–8.7ng) DYRK1B, active.**
5. Add 5.75µl of dH_2O.
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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DYRK1B Sequence Information

Protein human DYRK1B

Tags *N*-terminal GST

Native sequence M230 of the recombinant protein is equivalent to M1 of human DYRK1B

Accession number GenBank NM_004714

Recombinant DYRK1B 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 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFM AVPPGHGPFS
 241 GFPGPQEHTQ VLPDVRLLPR RLPLAFRDAT SAPLRKLSVD LIKTYKHINE VYYAKKKRRA
 301 QQAPPQDSSN KKEKKVLNHG YDDDNHDYIV RSGERWLERY EIDSLIGKGS FGQVVKAYDH
 361 QTQELVAIKI IKNKKAFLNQ AQIELRLLEL MNQHDTEMKY YIVHLKRHFM FRNHLCLVFE
 421 LLSYNLYDLL RNTHFRGVSL NLTRKLAQQL CTALLFLATP ELSIIHCDLK PENILLCNPK
 481 RSAIKIVDFG SSCQLGQRIY QYIQSRFYRS PEVLLGTPYD LAIDMWSLGC ILVEMHTGEP
 541 LFSGSNEVDQ MNRIVEVLGI PPAAMLDQAP KARKYFERLP GGGWTLRRTK ELRKDYQGPG
 601 TRRLQEVLGV QTGGPGGRRA GEPGHSPADY LRFQDLVLRM LEYEPAARIS PLGALQHGFF
 661 RRTADEATNT GPAGSSASTS PAPLDTCPSS STASSISSSG GSSGSSSDNR TYRYSNRYCG
 721 GPGPPITDCE MNSPQVPPSQ PLRPWAGGDV PHKTHQAPAS ASSLPGTGAQ LPPQPRYLGR
 781 PPSPTSPPPP ELMDVSLVGG PADCSPPHPA PAPQHPAASA LRTRMTGGRP PLPPPDDPAT
 841 LGPHLGLRGV PQSTAASS
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Recombinant DYRK1B nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
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 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
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 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggttccgc gtggatccaa ggaattcatg gccgtcccac cgggccatgg tcccttctct
 721 ggcttcccag ggccccagga gcacacgcag gtattgcctg atgtgcggct actgcctcgg
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1441 cgcagcgcca tcaagattgt ggacttcggc agctcctgcc agcttggcca gaggatctac
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1501 cagtatatcc agagccgctt ctaccgctca cctgaggtgc tcctgggcac accctacgac
1561 ctggccattg acatgtggtc cctgggctgc atccttgtgg agatgcacac cggagagccc
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2161 ggccctgggc cccctatcac agactgtgag atgaacagcc cccaggtccc accctcccag
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Reviewed and approved by site quality representative.