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Certificate of Analysis

DYRK1A, active
(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-951, 14-951-K, 14-951M

Parent Lot # WAB0497

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 DYRK1A, full length, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose and gel filtration.
Purity 74% by SDS-PAGE and Coomassie blue staining. MW = 112kDa.

Specific Activity (Parent lot# WAB0497): 1924U/mg, where one unit of DYRK1A, active activity is defined as 1nmol phosphate incorporated into 50µM (RRRFRPASPLRGPPK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.15mg/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.

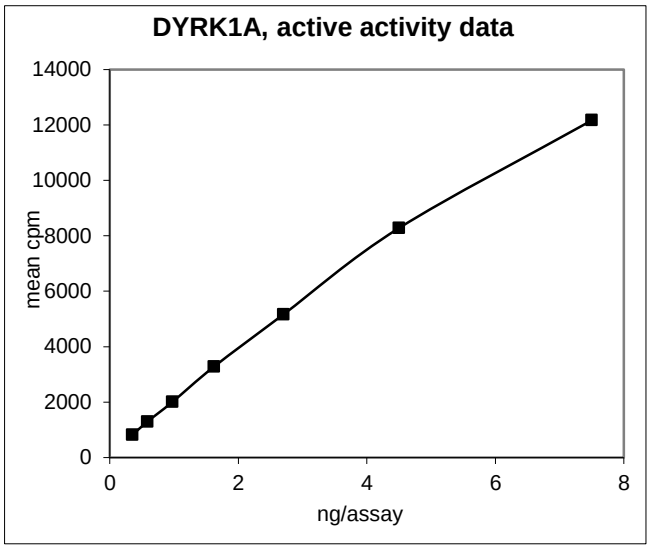
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

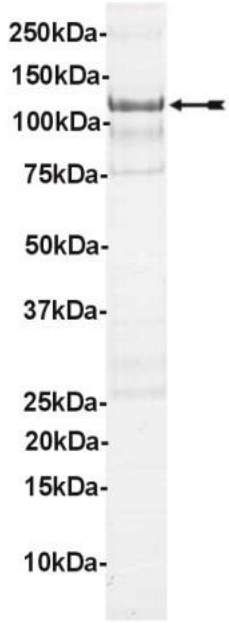
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.35–7.5ng 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 DYRK1A 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 active DYRK1A, 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. **DYRK1A, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.35–7.5ng per assay point.
4. **[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 0.5µl of **(RRRFRPASPLRGPPK)**.
3. Add **2.5µl (0.35–7.5ng) DYRK1A, active.**
4. Add 7µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl 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 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 1µl of 30% phosphoric acid.

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DYRK1A Sequence Information

Protein human DYRK1A

Tags *N*-terminal GST

Native sequence M236 of the recombinant protein is equivalent to M1 of human DYRK1A

Accession number GenBank NM_130436.2

Recombinant DYRK1A 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 LVPRGSKEFK GLRRQMHTGG
 241 ETSACKPSSV RLAPSFSFHA AGLQMAGQMP HSHQYSDRRQ PNISDQQVSA LSYSDQIQQP
 301 LTNQRRMPQT FRDPATAPLR KLSVDLIKTY KHINEVYYAK KKRRHQQGQG DDSSHKKERK
 361 VYNDGYDDDN YDYIVKNGEK WMDRYEIDSL IGKGSFGQVV KAYDRVEQEW VAIKIIKNKK
 421 AFLNQAQIEV RLLELMNKHD TEMKYYIVHL KRHFMFRNHL CLVFEMLSYN LYDLLRNTNF
 481 RGVSLNLTRK FAQQMCTALL FLATPELSII HCDLKPENIL LCNPKRSAIK IVDFGSSCQL
 541 GQRIYQYIQS RFYRSPEVLL GMPYDLAIDM WSLGCILVEM HTGEPLFSGA NEVDQMNKIV
 601 EVLGIPPAHI LDQAPKARKF FEKLPDGTWN LKKTKDGKRE YKPPGTRKLH NILGVETGGP
 661 GGRRAGESGH TVADYLKFKD LILRMLDYDP KTRIQPYYAL QHSFFKKTAD EGTNTSNSVS
 721 TSPAMEQSQS SGTTSSTSSS SGGSSGTSNS GRARSDPTHQ HRHSGGHFTA AVQAMDCETH
 781 SPQVRQQFPA PLGWSGTEAP TQVTVETHPV QETTFHVAPQ QNALHHHHGN SSHHHHHHHH
 841 HHHHHGQQAL GNRTRPRVYN SPTNSSSTQD SMEVGHSHHS MTSLSSSTTS SSTSSSSTGN
 901 QGNQAYQNRP VAANTLDFGQ NGAMDVNLTV YSNPRQETGI AGHPTYQFSA NTGPAHYMTE
 961 GHLTMRQGAD REESPMTGVC VQQSPVASS
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Recombinant DYRK1A nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 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
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggttccgc gtggatccaa ggaattcaaa ggcctacgtc gacaaatgca tacaggagga
 721 gagacttcag catgcaaacc ttcatctgtt cggcttgcac cgtcattttc attccatgct
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1621 gggcagagga tataccagta tattcagagt cgcttttatc ggtctccaga ggtgctactg
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Reviewed and approved by site quality representative.