

Certificate of Analysis

DYRK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-669, 14-669-K, 14-669M

Parent Lot # 30999U

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 6His-tagged, recombinant, full length human DYRK2, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 60.4% by SDS-PAGE and Coomassie blue staining. MW = 63.5kDa.

Specific Activity (Parent lot# 30999U): 245U/mg, where one unit of DYRK2, active activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.995mg/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.

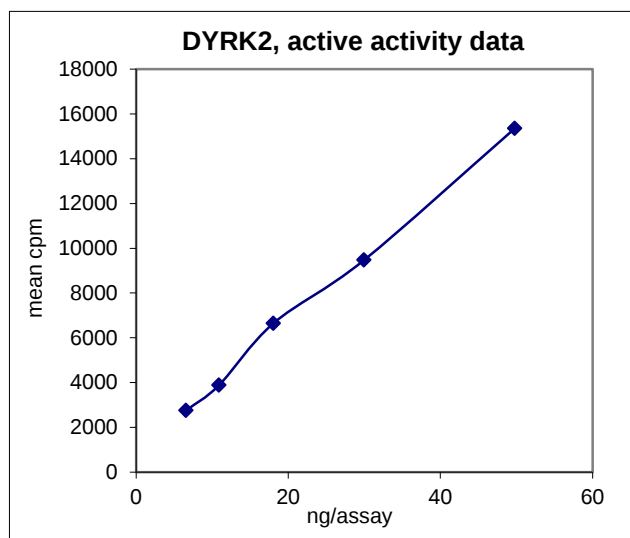
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

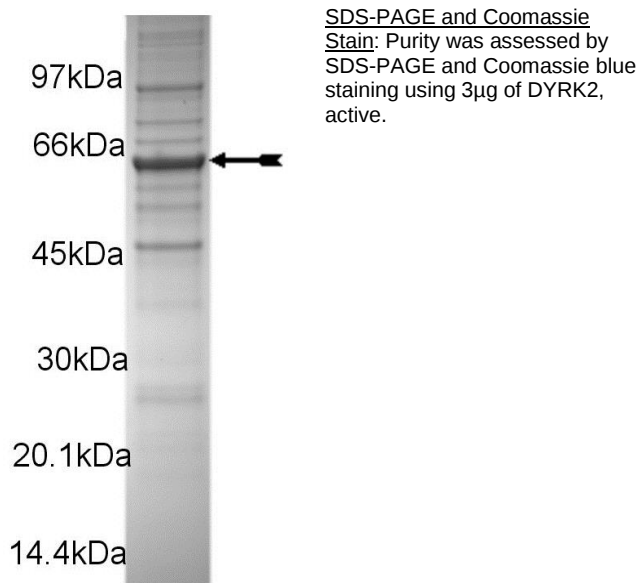
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 6.6–49.8ng of this lot of enzyme phosphorylated 2mg/ml casein 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 product identity as DYRK2 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **Casein:** Use at a final concentration of 2mg/ml. Make a 20mg/ml stock. Use 2.5µl per assay point.
3. **DYRK2, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6.6–49.8ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **casein**.
3. Add **2.5µl (6.6–49.8ng) DYRK2, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]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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DYRK2 Sequence Information

Protein	Human DYRK2
Tags	N-terminal 6His
Native sequence	M31 of the recombinant protein is equivalent to M1 of human DYRK2
Accession number	GenBank NM_003583

Recombinant DYRK2 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF MNDHLHVGSH AHGQIQVQQL FEDNSNKRTV
61 LTTQPNGLTT VGKTLGPVVP ERQLDSIHRR QGSSTSLKSM EGMGKVKATP MTPEQAMKQY
121 MQKLTAFEHH EIFSYPEIYF LGLNAKKRQG MTGGPNNGGY DDDQGSYVQV PHDHVAYRYE
181 VLKVIKGKSF GQVVKAYDVK VHQHVALKVM RNEKRFHRQA AEEIRILEHL RKQDKDNTMN
241 VIHMLENFTF RNHICMTFEL LSMNLYELIK KNKFQGFSLP LVRKFAHSIL QCLDALHKNR
301 IHCCLKPEN ILLKQQGRSG IKVIDFGSSC YEHQRVYTYI QSRFYRAPEV ILGARYGMPI
361 DMWSLGCILA ELLTGYP LLP GEDEGDLAC MIELLGMP SQ KLLDASKRAK NRVSSKGYPR
421 YCTVTTLSDG SVVLNGGRSR RGKLRGPPEP REWGNALKGC DDPLFLDFLK QCLEWDPVAVR
481 MTPGQALRHP WLRRLPKPP TGEKTSVKRI TESTGAITSI SKLPPSSSA SKLRTNLAQM
541 TDANGNIQQR TVLPKLVS

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Recombinant DYRK2 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
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121 gtcacggac agatccagg tcaacagttg tttgaggata acagtaacaa gcggacagtg
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