

Certificate of Analysis

DMPK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-649, 14-649-K, 14-649M

Parent Lot # 28641U

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, human DMPK, amino acids 1–549, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Purity 98% by SDS-PAGE and Coomassie blue staining. MW = 65kDa.

Specific Activity (Parent lot# 28641U): 12.3U/mg, where one unit of DMPK, active activity is defined as 1nmol phosphate incorporated into 250µM (KKSREGDYMTMQIG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.40mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamide, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

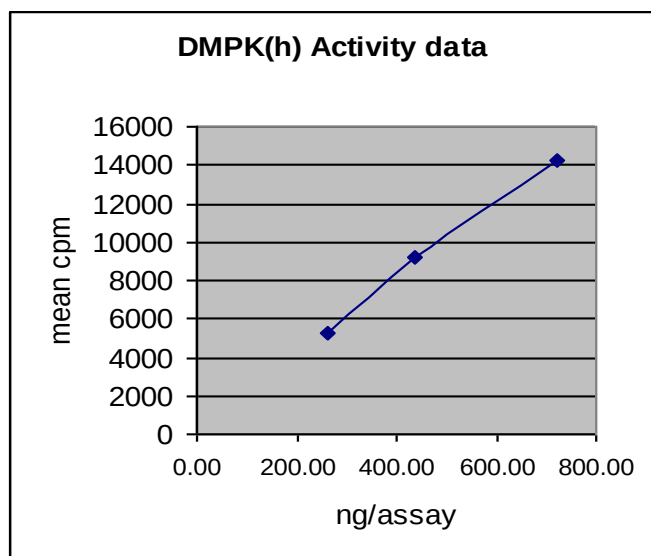
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

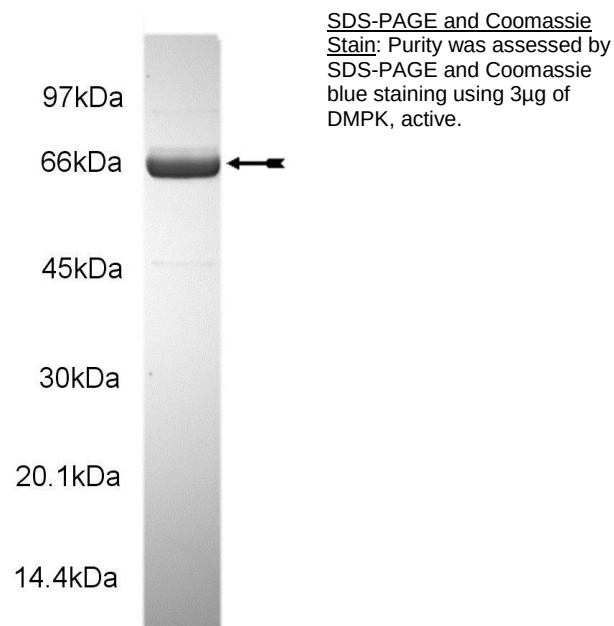
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 262–723ng of this lot of enzyme phosphorylated 250µM (KKSREGDYMTMQIG) 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 DMPK 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. **(KKSREGDYMTMQIG):** Use at a final assay concentration of 250µM. Make a 2.5mM stock. Add 2.5µl of stock per assay point.
3. **Triton X-100:** Use at a final assay concentration of 1%. Make up a 10% stock. Add 2.5µl of stock per assay point.
4. **DMPK, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 262–723ng per assay point.
5. **[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 **(KKSREGDYMTMQIG)**.
3. Add 2.5µl of 10% Triton X-100.
4. Add **2.5µl (262–723ng) DMPK, active**.
5. Add 2.5µl of dH₂O.
6. Add 10µl of diluted [γ-³³P]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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DMPK Sequence Information

<u>Protein</u>	Human DMPK
<u>Tags</u>	N-Terminal 6His
<u>Native sequence</u>	M31 of the recombinant protein is equivalent to M1 of native human DMPK
<u>Accession number</u>	GenBank NM_004409

Recombinant DMPK amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MSAEVLRLRL QQLVLDPGFL GLEPLDLLL
61 GVHQELGASE LAQDKYVADF LQWAEPIVVR LKEVRLQRDD FEILKVIGRG AFSEVAVVKM
121 KQTGQVYAMK IMNKWMLKR GEVSCFREER DVLVNGDRRW ITQLHFAFQD ENYLYLVMY
181 YVGDDLTLTLL SKFGERIPAE MARFYLAIEV MAIDSVHRLG YVHRDIKPDN ILLDRCGHIR
241 LADFGSCLKL RADGTVRSLV AVGTPDYLSL EILQAVGGGP GTGSYGPECD WWALGVFAYE
301 MFYGGQTPFYA DSTAETYGKI VHYKEHLSLP LVDEGVPEEA RDFIQRLCLP PETRLGRGGA
361 GDFRTHPFFF GLDWDGLRDS VPPFTPDEFEG ATDTCNFDLV EDGLTAMVSG GGETLS DIRE
421 GAPLGVLHPF VGYSYSCMAL RDSEVPGPTP MELEAEQLLE PHVQAPSLEP SVSPQDETAE
481 VAVPAAVPAA EAEAEVTLRE LQEALEEEVL TRQSLREME AIRTDNQNFA SQLREAEARN
541 RDLEAHVRQL QERMELLQAE GATAVTGVPS PRATDPPSH

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Recombinant DMPK nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc atgtcagccg aggtgcggct gaggcggctc
121 cagcagctgg tgttgaccc gggcttcctg gggctggagc ccctgctcga ccttctcctg
181 ggcgtccacc aggagctggg cgcctccgaa ctggcccagg acaagtacgt ggccgacttc
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361 aagcagacgg gccaggtgta tgccatgaag atcatgaaca agtgggacat gctgaagagg
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1501 ctccaggaag ccctggagga ggaggtgctc acccggcaga gcctgagccg ggagatggag
1561 gccatccgca cggacaacca gaacttcgcc agtcaactac gcgaggcaga ggctcggaac

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1621 cgggacctag aggcacacgt ccggcagttg caggagcgga tggagttgct gcaggcagag
1681 ggagccacag ctgtcacggg ggtccccagt ccccgggcca cggatccacc ttcccattag
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