

Certificate of Analysis

MKP5, active

(Recombinant enzyme expressed in *E. coli* cells)

Item # 14-779, 14-779-K, 14-779M

Parent Lot # D7EN022N

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 MKP5, amino acids 320–end, expressed in *E. coli* cells. Purified using Ni²⁺/NTA agarose. Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 20.6kDa.

Specific Activity (Parent lot# D7EN022N): 1511U/mg, where one unit of MKP5, active activity is defined as the release of 1nmol of phosphate per minute from the phosphorylated substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) at room temperature.

Formulation: 2.16mg/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.

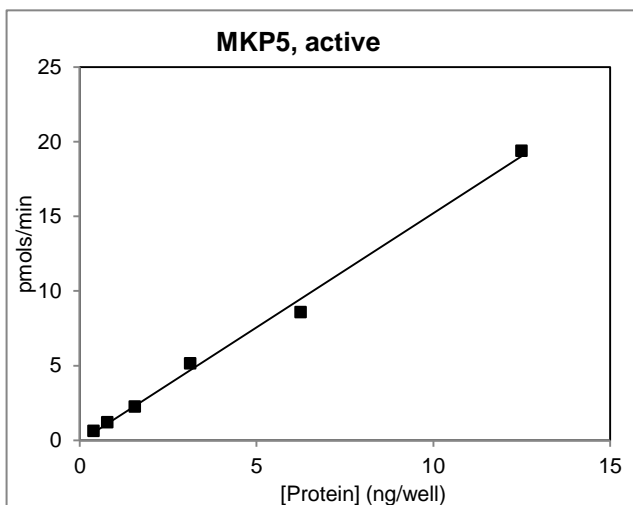
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

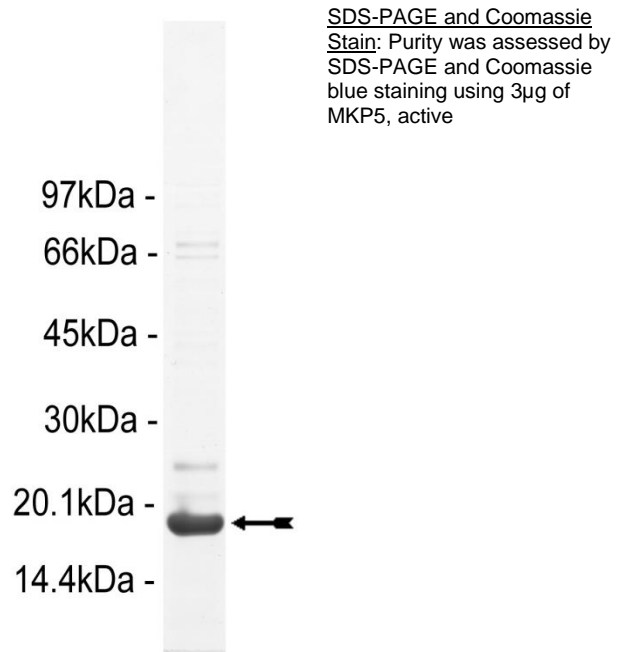
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Phosphatase Assay: 0.4–12.5ng of this lot of enzyme dephosphorylated 200µM DiFMUP in the assay described on page two. Assay background was subtracted from the actual Fluorescence Intensity (FI) to yield the results shown below. Quantification of FI was against a 6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU) standard curve.



MS Tryptic Fingerprint: Confirmed identity as MKP5 with the translated sequence listed on page three.



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Phosphatase Assay Protocol

Stock Solutions:

1. **Reaction Buffer:** 60mM Hepes pH7.2, 150mM NaCl, 1mM EDTA, 0.17mM DTT, 0.83 (v/v)% glycerol, 0.017 (w/v)% BSA, 0.002% Brij-35.
2. 500 μ M DiFMUP (Molecular Probes Catalogue# D6567) in water.
3. 100mM sodium orthovanadate.
4. 500 μ M DiFMU (Molecular Probes Catalogue# D6566) in water for the calibration curve.

Assay Procedure:

1. Dilute **MKP5** in reaction buffer and use 0.4–12.5ng in 15 μ l per assay point.
2. Add 10 μ l DiFMUP 500 μ M stock solution (200 μ M final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5 μ l of 100mM sodium orthovanadate.
5. Read FI using an appropriate reader (Excitation 340nm; Emission 450nm).
6. Subtract the zero enzyme values from each FI reading and calculate the enzyme activity by conversion to nmoles product formed using a DiFMU standard calibration curve.

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MKP5 Sequence Information

<u>Protein</u>	Human MKP5
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	A15 of the recombinant sequence is equivalent to A320 of human MKP5
<u>Accession number</u>	GenBank NM_007207

Recombinant MKP5 amino acid sequence:

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1 MHHHHHHENL YFQGAELTPI LPFLFLGNEQ DAQDLDTMQR LNIGYVINVT THLPLYHYEK
61 GLFNYKRLPA TDSNKQNLRLQ YFEEAFEFIE EAHQCGKGLL IHCQAGVSRS ATIVIAYLMK
121 HTRMTMTDAY KFKVKGKRPII SPNLNFMGQL LEFEEDLNNG VTPRILTPKL MGVETVV
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Recombinant MKP5 nucleotide sequence:

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1 atgcatcatc accatcacca tgaaaacctg tattttcagg gcgctgagct cccccccatc
61 ttgcccttcc tgttccttgg caatgagcag gatgctcagg acctggacac catgcagcgg
121 ctgaacatcg gctacgtcat caacgtcacc actcatcttc ccctctacca ctatgagaaa
181 ggctgttca actacaagcg gctgccagcc actgacagca acaagcagaa cctgcggcag
241 tactttgaag aggcttttga gttcattgag gaagctcacc agtgtgggaa ggggcttctc
301 atccactgcc aggctggggg gtcccgcctc gccaccatcg tcatcgctta cttgatgaag
361 cacactcgga tgaccatgac tgatgcttat aaatttgtca aaggcaaacg accaattatc
421 tccccaaacc ttaacttcat ggggcagttg ctagagtctg aggaagacct aaacaacggt
481 gtgacaccga gaatccttac accaaagctg atgggcgtgg agacggttgt gtga
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Reviewed and approved by site quality representative.

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