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Certificate of Analysis

DUSP22, active

(Recombinant enzyme expressed in *E. coli* cells)
Item # 14-641, 14-641-K, 14-641M
Parent Lot # 1922204

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 DUSP22, full length, expressed in *E. coli* cells. Purified using glutathione agarose.
Purity 91.9% by SDS-PAGE and Coomassie blue staining. MW = 47.3kDa.

Specific Activity (Parent lot# 1922204): 360U/mg, where one unit of DUSP22, 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: 1.629mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

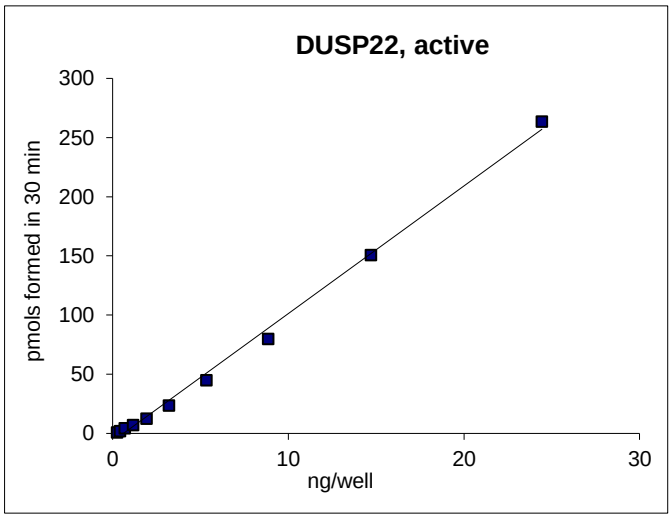
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

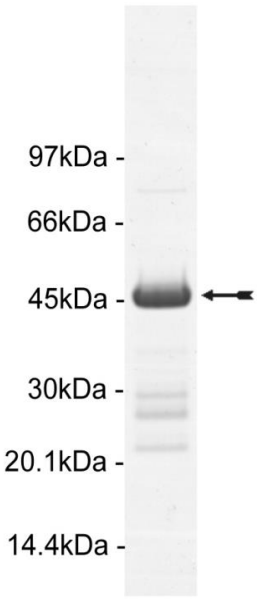
Quality Control Testing

Phosphatase Assay: 0.2–2.4ng 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 DUSP22 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 DUSP22, active.



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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. 500µM DiFMUP (Molecular Probes Catalogue# D6567) in water.
3. 100mM sodium orthovanadate.
4. 500µM DiFMU (Molecular Probes Catalogue# D6566) in water for the calibration curve.

Assay Procedure:

1. Dilute **DUSP22** in reaction buffer and use 0.2–2.4ng in 15µl per assay point.
2. Add 10µl DiFMUP 500 µM stock solution (200µM final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5µl 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.

Preparation of DiFMU Standard Calibration Curve

1. Prepare a series of standards according to the table below:

Volume of 100µM DiFMU (product)	Volume of 100µM DiFMUP (substrate)	pmoles DiFMU per well	Represents Substrate Conversion of:
0µl	100µl	0	0%
1µl	99µl	25	1%
2µl	98µl	50	2%
3µl	97µl	75	3%
4µl	96µl	100	4%
5µl	95µl	125	5%
8µl	92µl	200	8%
12µl	88µl	300	12%

2. Add 25µl of each prepared standard to triplicate wells and read fluorescence intensity using an appropriate reader (Excitation 358nm, Emission 455nm).

Note: Substrate conversion above 15% introduces the possibility of skewing the curve so that calculated specific activity will be less than actual specific activity. This substrate limitation kinetic varies with each enzyme and may be determined by plotting product formed per unit time versus enzyme concentration. At low enzyme concentrations, the rate of product formation will be proportional to the enzyme concentration used. This relationship plateaus at higher enzyme concentrations.

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DUSP22 Sequence Information

Protein Human DUSP22

Tags N-Terminal GST

Native sequence M227 of the recombinant protein is equivalent to M1 of human DUSP22

Accession number GenBank NM_020185.

Recombinant DUSP22 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 LVPRGSMGNG MNKILPGLYI
 241 GNFKDARDAE QLSKNKVTHI LSVHDSARPM LEGVKYLCIP AADSPSQNLT RHFKESIKFI
 301 HECRLRGESC LVHCLAGVSR SVTLVIAYIM TVTDFGWEDA LHTVRAGRSC ANPNVGFQRQ
 361 LQEFEKHEVH QYRQWLKEEY GESPLQDAEE AKNILAAPGI LKFWAFLRRL
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Recombinant DUSP22 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
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 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
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1021 ctgcacaccg tgcgtgctgg gagatcctgt gccaacccca acgtgggctt ccagagacag
1081 ctccaggagt ttgagaagca tgaggtccat cagtatcggc agtggctgaa ggaagaatat
1141 ggagagagcc ctttgcagga tgcagaagaa gccaaaaaca ttctggccgc tccaggaatt
1201 ctgaagttct gggcctttct cagaagactg taa
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