

Product Information

DUSP22, active, GST tagged, human recombinant, expressed in *E. coli* cells

Catalog Number **SRP5021**
Storage Temperature -70°C

Synonyms: VHX, JKAP, JSP1, MKPX

Product Description

The dual specificity phosphatases (DUSP) family, which can dephosphorylate both phosphoserine/threonine and phosphotyrosine residues, contains two subgroups: "typical" DUSP (include 11 MKPs that dephosphorylate the mitogen-activated protein kinases Erk, Jnk, and p38 at their dually phosphorylated TXY activation loop motif) and "atypical" DUSP (include 8 enzymes that lack specific MAP kinase-targeting motifs and tend to be much smaller proteins, less than 250 amino acid residues).¹ DUSP22, also called JKAP, JSP1, and MKPX plays a role in various inflammatory and proliferative disorders associated with dysfunctional Jnk signaling.²

Recombinant, full length, human DUSP22 was expressed in *E. coli* cells using an N-terminal GST tag. The gene accession number is NM_020185. Recombinant protein stored in 20 mM MOPS, pH 7.5, 50 mM NaCl, 10 mM glutathione, 0.25 mM DTT, 0.1 mM PMSF, 30% glycerol.

Molecular mass: ~43 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 510–690 nmol/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at -70°C is recommended. After opening, aliquot into smaller quantities and store at -70°C . Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

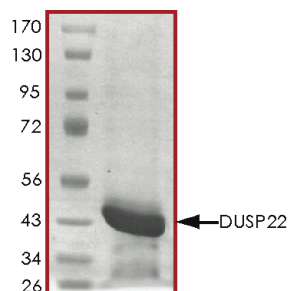
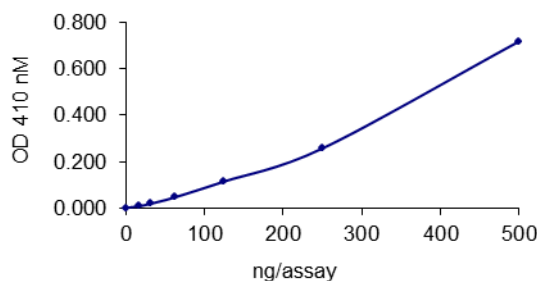


Figure 2.
Specific Activity of Typical Lot
510–690 nmol/min/mg



Procedure

Preparation Instructions

Phosphatase Assay Buffer – 125 mM HEPES, pH7.2, 250 mM NaCl, and 12.5 mM EDTA.

Phosphatase Dilution Buffer – Dilute the Phosphatase Assay Buffer 5-fold with a 5 mM DTT and 65 ng/μl BSA solution.

Phosphatase Solution – Dilute the active DUSP22 (0.1 μg/μl) with Phosphatase Dilution Buffer to the desired concentration.

Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active DUSP22 for optimal results.

Stopping Solution – 2 M NaOH

Substrate Stock Solution – Prepare 500 mM *p*-nitro-phenyl phosphate (pNPP) Substrate Stock Solution by dissolving 131.5 mg of pNPP in 1 ml of Phosphatase Dilution Buffer. Store at –20 °C. Avoid direct light exposure.

Substrate Assay Solution – Prepare 50 mM pNPP Substrate Assay Solution by diluting the Substrate Stock Solution ten fold with Phosphatase Dilution Buffer. Prepare fresh before assay.

Phosphatase Assay

1. Prepare sufficient Substrate Assay Solution.
2. Thaw the active DUSP22 and Phosphate Dilution Buffer on ice.
3. In a pre-cooled microcentrifuge tube, add the following reaction components:
 - 10 μl of Phosphatase Solution
 - 20 μl of 50 mM pNPP Substrate Assay solution
 - 170 μl of Phosphatase Dilution Buffer
4. Set up a blank control as outlined in step 3, substituting 10μl of Phosphatase Dilution Buffer for the Phosphatase Solution.
5. Initiate each reaction by incubating the mixture in a water bath at 37 °C for 20 minutes.
6. After the 20 minute incubation, stop the reaction by the addition of 50 μl of 2 M NaOH Stopping Solution.
7. Measure the absorbance of the reaction solution in a spectrophotometer at 405 nm.
8. Determine the Phosphatase specific activity.

Calculations:

Specific Phosphatase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{Pv \times OD_{405nm}}{\epsilon \times d \times T \times Pm}$$

- Pv - Phosphatase volume (μl)
ε - extinction coefficient (17.8 μl/nmole/cm)
d - pathlength of light (cm)
T - incubation time (min)
Pm - Phosphatase amount (mg)

References

1. Andres, A. et al., VHY, a novel myristoylated testis-restricted dual specificity protein phosphatase related to VHX. J. Biol. Chem., **279**, 32586-32591 (2004).
2. Shen, Y. et al., Activation of the Jnk signaling pathway by a dual-specificity phosphatase, JSP-1. Proc. Natl. Acad. Sci. U S A., **98**, 13613-13618 (2001).

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