

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-JOSD1

<u>Enzyme description:-</u>	His-JOSD1
<u>Clone number:-</u>	SC20956
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -Sephadex
<u>Expression level:-</u>	7 mg/L

Calculated molecular mass:-

Monoisotopic	25625 Da
Average Mass	25641 Da
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 9.13

Purity:- 95%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80°C

Assay:-

Ub-Rho110-Gly cleavage assay monitored by Ex/Em 485/535 nm

Assay buffer:-

40 mM Tris pH 7.5, 100 mM NaCl, 5 mM DTT, 0.01% Triton X-100, 0.005% Ovalbumin, 0.5 μM Ub-Rho110-Gly

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Clone Data Sheet

His-JOSD1

<u>Protein</u>	His-JOSD1
<u>Synonyms</u>	Josephin-1, JSPH1
<u>Clone Number</u>	SC20956
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q15040 DNA: NM_014876.5
<u>Tags</u>	N-terminal His ₆ tag
<u>Amino acid sequence of expressed protein</u>	MGSSHHHHHSSGLEVL<u>FQGP</u>SGSMSCVPWKGDKAKSESLELPQAAPPQIY HEKQRRELCALHALNNVFQDSNAFTRDTLQEIFQRLSPNTMVTPHKKSML GNGNYDVNVIMAAALQTKGYEAVWWDKRRDVGVIALTNVMGFIMNLPSSLC WGPLKLPLKRQHWICVREVGGAYYNLDSKLMPEWIGGESELRKFLKHHL RGKNCELLLVVPEEVEAHQSWRTDV
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	Precision site underlined
<u>Cloning sites</u>	BamH1 / Not1

**DNA sequence of
insert**

ATGAGTTGTGTGCCATGGAAAGGAGACAAGGCCAAATCTGAATCATTGGA
GCTGCCCCAGGCAGCACCCCCACAAATCTACCATGAGAAACAGCGCAGGG
AGCTTTGTGCCCTCCACGCCCTCAATAACGTCTTCCAGGACAGCAATGCC
TTCACCCGGGATACGCTGCAAGAGATTTTCCAGAGGTTGTCTCCAAACAC
CATGGTGACACCTCACAAGAAGAGCATGCTGGGAAATGGCAACTACGATG
TGAATGTCATTATGGCAGCACTTCAGACCAAAGGCTATGAAGCTGTTTGG
TGGGACAAGCGCAGGGATGTCGGTGTTCATTGCCCTCACTAACGTCATGGG
CTTCATCATGAATCTGCCCTCCAGCCTATGCTGGGGTCCACTGAAACTGC
CCCTCAAAGGCAGCACTGGATCTGTGTTTCGAGAGGTGGGAGGGGCCTAC
TACAACCTCGACTCCAAACTCAAGATGCCCGAGTGGATTGGAGGCGAGAG
CGAGCTCAGGAAGTTTCTAAAACATCATTGCGAGGAAAGAACTGTGAAC
TCCTGCTGGTGGTACCAGAAGAGGTAGAGGCTCATCAGAGTTGGAGGACC
GATGTGTAA