Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-JOSD2

Enzyme description:- His-JOSD2

Clone number:- DU20941

Source:- BL21 Recombinant

Tag:- His

Purification method:- N-terminal His_6-tag

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic	23186 Da
Average Mass	23199 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.35

Purity:- 60%

Enzyme storage buffer:-

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

Storage temperature:- -80ºC

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-JOSD2

Protein	His-JOSD2
Synonyms	Josephin-2
Clone Number	DU20941
Species	Human
Accession Number	Protein: Q8TAC2 DNA: NM_138334.2
Tags	N-terminal His_6-tag
Amino acid sequence of expressed protein	MGSSHHHHHHSSGLEVLFQGPGS**MSQAPGAQPSPPTVYHERQRLELCAVH ALNNVLQQQLFSQEAADEICKRLAPDSRLNPHRSLLGTGNYDVNVIMAAL QGLGLAAVWWDRRRPLSQLALPQVLGLILNLPSPVSLGLLSLPLRRRHWV ALRQVDGVYYNLDSKLRAPEALGDEDGVRAFLAAALAQGLCEVLLVVTKE VEEKGSWLRTD**
Native sequence	in bold
Protease cleavage	Precission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

GGATCCATGTCCCAGGCCCCGGGAGCACAGCCGAGCCCACCCACCGTGTA
CCACGAACGGCAGCGCCTGGAGCTGTGTGCTGTCCACGCCCTCAACAACG
TTCTGCAGCAGCAGCTCTTTAGCCAGGAGGCTGCCGATGAGATCTGCAAG
AGGTTGGCCCCAGACTCCCGGCTGAACCCTCATCGCAGCCTCCTGGGCAC
CGGCAACTATGATGTCAATGTGATCATGGCCGCTCTGCAGGGGCTGGGCC
TGGCCGCCGTGTGGTGGGACAGGAGGAGGCCCCTGTCCCAGCTGGCCCTG
CCCCAGGTACTGGGGCTGATCCTGAACCTGCCCTCGCCCGTGTCGCTGGG
GCTGCTGTCACTGCCGCTGCGCCGGCGGCACTGGGTGGCCCTGCGCCAGG
TGGACGGTGTCTACTACAACCTGGACTCCAAGCTGCGGGCGCCCGAGGCC
CTGGGGGGATGAGGACGGAGTCAGGGCCTTCCTGGCGGCTGCGCTGGCCCA
GGGCCTGTGCGAGGTGCTGCTGGTAGTGACCAAGGAGGTGGAGGAGAAGG
GCAGCTGGCTGCGGACAGACTGAGCGGCCGC