Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-USP4

Enzyme description:- USP4

Clone number:- DU14350

Source:- BL21 Recombinant

Tag:- N-terminal His_6 tag

Purification method:- Ni^{++}-Sepharose, IEX

Expression level:- 4 mg/L

Calculated molecular mass:-

Monoisotopic	110909 Da
Average Mass	111006 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.26

Purity:- 90%

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-USP4

Protein	USP4
Synonyms	UNP
Clone Number	DU14350
Species	Human
Accession Number	Protein: Q13107 DNA: NM_003363
Tags	N-terminal His_6 tag
Amino acid sequence of expressed protein	MGSSHHHHHHSSGLEVLFQGPGS**MAEGGGCRERPDAETQKSELGPLMRTT LQRGAQWYLIDSRWFKQWKKYVGFDSWDMYNVGEHNLFPGPIDNSGLFSD PESQTLKEHLIDELDYVLVPTEAWNKLLNWYGCVEGQQPIVRKVVEHGLF VKHCKVEVYLLELKLCENSDPTNVLSCHFSKADTIATIEKEMRKLFNIPA ERETRLWNKYMSNTYEQLSKLDNTVQDAGLYQGQVLVIEPQNEDGTWPRQ TLQSKSSTAPSRNFTTSPKSSASPYSSVSASLIANGDSTSTCGMHSSGVS RGGSGFSASYNCQEPPSSHIQPGLCGLGNLGNTCFMNSALQCLSNTAPLT DYFLKDEYEAEINRDNPLGMKGEIAEAYAELIKQMWSGRDAHVAPRMFKT QVGRFAPQFSGYQQQDSQELLAFLLDGLHEDLNRVKKKPYLELKDANGRP DAVVAKEAWENHRLRNDSVIVDTFHGLFKSTLVCPECAKVSVTFDPFCYL TLPLPLKKDRVMEVFLVPADPHCRPTQYRVTVPLMGAVSDLCEALSRLSG IAAENMVVADVYNHRFHKIFQMDEGLNHIMPRDDIFVYEVCSTSVDGSEC VTLPVYFRERKSRPSSTSSASALYGQPLLLSVPKHKLTLESLYQAVCDRI SRYVKQPLPDEFGSSPLEPGACNGSRNSCEGEDEEEMEHQEEGKEQLSET EGSGEDEPGNDPSETTQKKIKGQPCPKRLFTFSLVNSYGTADINSLAADG KLLKLNSRSTLAMDWDSETRRLYYDEQESEAYEKHVSMLQPQKKKKTTVA LRDCIELFTTMETLGEHDPWYCPNCKKHQQATKKFDLWSLPKILVVHLKR FSYNRYWRDKLDTVVEFPIRGLNMSEFVCNLSARPYVYDLIAVSNHYGAM GVGHYTAYAKNKLNGKWYYFDDSNVSLASEDQIVTKAAYVLFYQRRDDEF YKTPSLSSSGSSDGGTRPSSSQQGFGDDEACSMDTN**
Native sequence	in bold
Protease cleavage	Precission site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

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