

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of USP7

<u>Enzyme description:-</u>	USP7 C223A
<u>Clone number:-</u>	DU30030
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -Sephrose, SEC
<u>Expression level:-</u>	10 mg/L

Calculated molecular mass:-

Monoisotopic	130601 Da
Average Mass	130682 Da
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 5.96

Purity:- 80%

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-USP7 C223A

<u>Protein</u>	USP7 C223A
<u>Synonyms</u>	HAUSP
<u>Clone Number</u>	DU30030
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q93009 DNA: NM_003470.2
<u>Tags</u>	N-terminal His ₆ tag
<u>Amino acid sequence of expressed protein</u>	<p>MGSSHHHHHSSGLEVLFGPGSMNHQOQOQOQKAGEQQLSEPEDMEMEA GDTDDPPRITQNPVINGNVALSDGHNTAEEDMEDDTSWRSEATFQFTVER FSRLSESVLSPPCFVRNLPWKIMVMPRFYPDRPHQKSVGGFFLQCNAESDS TSWSCHAQAVLKI INYRDEKSF SRRISHLFFHKENDWGF SNFMAWSEVT DPEKGFIDDDKVTFEVQADAPHGVAWDSKKHTGYVGLKNOGATAYMNS LLQTLFFTNQLRKAVYMPTEGDDSSKSVPLALQRFVYELQHSDKPVGTK KLTKSFGWETLDSFMQHDVQELCRVLLDNVENKMKGTCVEGTIPKLFGRK MVSYIQCKEVDYRSDRREDYDIQLSIKGGKNIFESFVDYVAVEQLDGDN KYDAGEHGLQEAKEGKFLTLPPVLHLQLMRFMYDPQTDQNIKINDRFEF PEQLPLDEFLOKTDPKDPANYILHAVLVHSGDNHGGHYVVYLNPKGDGKW CKFDDDVSRCTKEEAI EHNYYGGHDDLSVRHCTNAYMLVYI RESKLSEV LQAVTDHDIPOQLVERLQEEKRIEAQKRKERQEAHLYMQVQIVAEDQFCG HOGNDMYDEEKVKYTVFKVLKNSSLAEFVQSLSQTMGFPQDQIRLWPMQA RSNGTKRPAMDNEADGNKTMIELSDNENPWTIFLETVDPELAASGATLP KFDKDHVMLFLKMYDPKTRSLNYCGHIYTPISCKIRDLLPVMCDRAGFI QDTSILILYEEVKPNLTERIQDYDVSLDKALDELMDGDIIVFQKDDPENDN SELPTAKEYFRDLYHRVDVIFCDKTI PNDPGFVVTL SNRMNYFQVAKTVA QRLNTDPMLLQFFKSOGYRDGPGNPLRHNYEGTLRDLLOFFKPRQPKKLY YQQLKMKITDFENRRSFKCIWLNSQFREEEITLYPDKHGCVRDLLLEECKK AVELGEKASGKLRLLIIVSYKIGVHQEDELLECLSPATSRTRFRIEIIPL DQVDIDKENEMLVTVAHFHKEVFGTFGIPFLLRIHQGEHFREVMKRIQSL LDIQEKEFEKFKFAIVMTGRHQYINEDYEYVNLKDFEPQPGNMSHPRPWL GLDHFNKAPKRSRYTYLEKAIKIHN</p>
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	Precision site underlined
<u>Cloning sites</u>	BamH1 / Not1
<u>DNA sequence of insert</u>	<p>GGATCCATGAACCACCAGCAGCAGCAGCAGCAGCAGAAAAGCGGGCGAGCA GCAGTTGAGCGAGCCCGAGGACATGGAGATGGAAGCGGGAGATACAGATG ACCCACCAAGAATTACTCAGAACCCTGTGATCAATGGGAATGTGGCCCTG AGTGATGGACACAACACCGCGGAGGAGGACATGGAGGATGACACCAGTTG GCGCTCCGAGGCAACCTTTCAGTTCACCTGTGGAGCGCTTCAGCAGACTGA GTGAGTCGGTCCCTTAGCCCTCCGTGTTTTGTGCGAAATCTGCCATGGAAG ATTATGGTGATGCCACGCTTTTATCCAGACAGACCACACCAAAAAGCGT AGGATTCCTTCTCCAGTGCAATGCTGAATCTGATTCACGTCATGGTCTT GCCATGCACAAGCAGTGCTGAAGATAATAAATTACAGAGATGATGAAAAG TCGTCAGTCGTCGTATTAGTCATTTGTTCTTCATAAAGAAAATGATTG GGGATTTTCCAATTTTATGGCCTGGAGTGAAGTGACCGATCCTGAGAAAAG</p>

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