

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-DEN1 C163A [Δ 93-99]

Enzyme description:- GST-DEN1 C163A [Δ 93-99]

Clone number:- DU20824

Source:- BL21 Recombinant

Tag:- GST

Purification method:- GSH-Sepharose

Expression level:- 4 mg/L

Calculated molecular mass:-

Monoisotopic 50892 Da

Average Mass 50924 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.65

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 μ M Ub-Rho110-Gly

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Clone Data Sheet

GST-DEN1 C163A [Δ 93-99]

<u>Protein</u>	GST-DEN1 C163A [Δ 93-99]
<u>Synonyms</u>	SEN8, NEDP1, PRSC2
<u>Clone Number</u>	DU20824
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q96LD8 DNA: NM_145204.3
<u>Tags</u>	N-terminal GST tag
<u>Amino acid sequence of expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL <u>FQG</u> PLGSPNSRVDMDP <u>VVLSY</u> MDSLLRQSDVSLLDPPSWLNDHIIGFAFEYFANSQFHDCSDHVSFISPEVTQFIKCTSNPAEIAMFLEPLDLPNKRVVFLAINDGTHWSLLVYLQDKNSFFHYDHSRSNSVHAKQVAEKLEAFLGRKGDKLAFVEEKAPAQONSYDAGMYVICNTEALCQNFRRQQTESLLQLLTPAYITKKRGEWKDLITTLAKK
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	Precision site underlined
<u>Cloning sites</u>	Sall / Not1
<u>DNA sequence of insert</u>	GTCGACATGGACCCCGTAGTCTTGAGTTACATGGACAGTCTACTGCGGCAATCAGATGTCTCACTATTGGATCCGCCAAGCTGGCTCAATGACCATATTATTGGGTTTGCGTTTGAGTACTTTGCCAACAGTCAGTTTCATGACTGCTCTGATCACGTCAGTTTCATCAGCCCTGAAGTCACCCAGTTCATCAAGTGCCTAGCAACCCAGCAGAGATTGCCATGTTCCCTGAACCACTGGACCTCCCCAACAAAGAGAGTTGTATTTTTAGCCATCAATGATGGAACCACTGGAGTTTATTGGTCTACCTCCAAGATAAAAATAGCTTTTTTTCATTATGATTCCCATAGCAGGAGCAACTCAGTTCACGCAAAGCAGGTAGCAGAGAACTGGAGGCTTCTTAGGCAGAAAAGGAGACAAACTGGCCTTTGTGGAAGAGAAAAGCCCTGCCAACAAAACAGCTATGACGCTGGATGTACGTGATATGTAACACTGAGGCCTTGTGTGTCAGAACTTCTTTAGGCAACAGACAGAATCACTGCTGCAGCTACTCACCCCTGCATACATCACAAAGAAGAGGGGAGAATGGAAAAGATCTCATTACCACACTTGCTAAAAAGTAGGCGGCCGC