

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of DAZAP T315A [2 - 407]

Enzyme description:- DAZAP T315A [2 - 407]

Clone number:- DU 3218

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 70,001.64 daltons

Average Mass 70,046.34 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.87

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

DAZAP T315A [2 - 407]

<u>Protein</u>	DAZAP T315A [2 - 407]
<u>Clone number</u>	DU 3267
<u>Species</u>	Human
<u>Accession number</u>	NM_018959.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSNNSGADEIGKLFVG GLDWSTTQETLRSYFSQYGEVVDCVIMKDKT TNQSRGFGFVKFKDPNCV GTVLASRPHTLDGRNIDPKPCTPRGMQPERTRPKEGWQKGPRSDNSKSN KIFVGGI PHNCGETELREYFKKF GVVTEVVM IYDAEKQRPRGFGF ITFE DEQSV DQAVNMHFHDIMGKKVEVKRAEPRDSKSQAPGQPGASQWGSRVV PNAANGWAGQPPPTWQOGYGPOGMWVPAGQAIGGYGPPPPAGRGAPPPP PFTSYIVSTPPGGFPPPQGFPOGYGAPPQFSFGYGPPPPPPDQFAPPGV PPPPA APGAAPLAFPPPPSQAAPDMSKPPTAQPDPYPYGQYAGYGQDLSG FGQGFSDPSQPPPSYGGPSVPGSGGPPAGGSGFGRGQNHNVQGFHPYRR</p>
<u>Native sequence</u>	<p>Amino acids N2 – R407 (end) of human DAZAP. Residue N232 of the fusion protein is equivalent to N2 of the native enzyme. The GST tag is located at residues 1 – 220. The protein has a T315A mutation. Residue T315 is equivalent to residue A545 of the fusion protein.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 sites of pGEX6P-1

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Nucleotide
Sequence of insert

ggatccAACAAC TCGGGCGCCGACGAGATCGGGAAGCTCTTCGTGGGCGGTCTT
GACTGGAGCACGACCCAAGAGACTCTGCGCAGCTACTTTTCCCAATATGGAGAA
GTCGTAGATTGTGTTATCATGAAAGATAAAACCACCAACCAGTCTCGAGGCTTT
GGGTTTGTCAAATTTAAAGACCCAAACTGTGTGGGGACGGTGCTGGCCAGCAGA
CCGCACACGCTAGATGGCCGAAACATCGACCCCAAGCCATGCACACCCCGGGGG
ATGCAGCCGGAGAGAACACGGCCGAAGGAAGGATGGCAGAAAGGACCCAGGAGC
GATAACAGTAAATCAAATAAGATATTTGTTCGGTGGAAATTCCTCACAAATTGTGGT
GAGACAGAGCTCAGGGAATACTTCAAGAAGTTCGGAGTGGTCACGGAGGTAGTC
ATGATCTATGACGCCGAGAAGCAGAGGCCCCGAGGTTTTGGATTTATTACTTTT
GAGGACGAACAATCAGTGGACCAGGCTGTCAACATGCATTTTTCACGACATCATG
GGCAAAAAGTGAAGTTAAACGAGCTGAGCCTCGGGACAGCAAGAGCCAAGCG
CCGGGACAGCCAGGTGCCAGCCAGTGGGGGAGCCGGGTTGTGCCAACGCTGCC
AATGGCTGGGCAGGCCAGCCCCCGCCACGTGGCAGCAAGGATATGGCCCGCAA
GGAATGTGGGTGCCGGCAGGACAGGCGATTGGTGGCTATGGACCCGCCCTGCA
GGAAGAGGAGCCCCCGCCACCCACCGTTCACCTCCTACATCGTGTCCACC
CCTCCTGGAGGCTTTCCCCCTCCCCAGGGCTTCCCTCAGGGCTACGGTGCCCCG
CCACAGTTCAGTTTTGGCTACGGGCCCTCCACCTCCACCGCCAGATCAGTTTGCC
CCTCCGGGGTTCTCTCCACCAGCCGCTCCCGGGGCAGCACCTCTGGCTTTC
CCACCGCTCCGTCTCAGGCTGCCCCGGACATGAGCAAGCCCCCGACAGCTCAG
CCAGACTTCCCCTATGGTCAGTATGCAGGTTACGGGCAGGACTTGAGTGGCTTC
GGACAGGGCTTCTCAGACCCAGCCAGCAGCCTCCTTCTACGGGGTCCCTCC
GTGCCAGGGTCGGGGGGCCCCCGCCGGCAGCGGCTTTGGACGAGGGCAG
AACCACAACGTGCAAGGTTCCACCCCTACCGACGctaggatcc