

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of DAZAP [2 - 407]**

**Enzyme description:-** DAZAP [2 - 407]

**Clone number:-** DU 3123

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 70, 031.65 daltons

Average Mass 70, 076.36 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 [2 - 407]**

**Protein** DAZAP [2 - 407]

**Clone number** DU 3123

**Species** Human

**Accession number** NM\_018959

**Tags** N-terminal GST

**Bacterially expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG  
LEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA  
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH  
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS  
KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSNNSGADEIGKLFVG  
**GLDWSTTQETLRSYFSQYGEVVDCVIMKDKT TNQSRGFGFVKFKDPNCV**  
**GTVLASRPHTLDGRNIDPKPCTPRGMQPERTRPKEGWQKGPRSDNSKSN**  
**KIFVGGIPHNCGETELREYFKKFGVVTEVVM IYDAEKQRPRGFGFITFE**  
**DEQSV DQAVNMHFHDIMGKKVEVKRAEPRDSKSQAPGQPGASQWGSRVV**  
**PNAANGWAGQPPPTWQQGYGPQGMWVPAGQAIGGYGPPPPAGRGAPPPP**  
**PFTSYIVSTPPGGFPPPQGFPOGYGAPPQFSFGYGPPPPPPDQFAPPGV**  
**PPPPATPGAAPLAFPPPPSQAAPDMSKPPTAQPDFPYGQYAGYGQDLSG**  
**FGQGFSDPSQOPPSYGGPSVPGSGGPPAGGSGFGRGQNHNVQGFHPYRR**

**Native sequence** 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.

**Protease cleavage** PreScission (LEVLFQGP) residues 221 - 228

**Cloning sites** *Bam*H1 sites of pGEX6P-1

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**Nucleotide**  
**Sequence of insert**

ggatccAACAACTCGGGCGCCGACGAGATCGGGAAGCTCTTCGTGGGCG  
GTCTTGACTGGAGCACGACCCAAGAGACTCTGCGCAGCTACTTTTCCCA  
ATATGGAGAAGTCGTAGATTGTGTTATCATGAAAGATAAAACCACCAAC  
CAGTCTCGAGGCTTTGGGTTTGTCAAATTTAAAGACCCAAACTGTGTGG  
GGACGGTGCTGGCCAGCAGACCGCACACGCTAGATGGCCGAAACATCGA  
CCCCAAGCCATGCACACCCCGGGGGATGCAGCCGGAGAGAACACGGCCG  
AAGGAAGGATGGCAGAAAGGACCCAGGAGCGATAACAGTAAATCAAATA  
AGATATTTGTCTGGTGAATTCCTCACAATTGTGGTGAGACAGAGCTCAG  
GGAATACTTCAAGAAGTTCGGAGTGGTCACGGAGGTAGTCATGATCTAT  
GACGCCGAGAAGCAGAGGCCCGAGGTTTTGGATTTATTACTTTTCGAGG  
ACGAACAATCAGTGGACCAGGCTGTCAACATGCATTTTCACGACATCAT  
GGCAAAAAAGTGGAAGTTAAACGAGCTGAGCCTCGGGACAGCAAGAGC  
CAAGCGCCGGGACAGCCAGGTGCCAGCCAGTGGGGGAGCCGGGTTGTGC  
CCAACGCTGCCAATGGCTGGGCAGGCCAGCCCCCGCCACGTGGCAGCA  
AGGATATGGCCCGCAAGGAATGTGGGTGCCGGCAGGACAGGCGATTGGT  
GGCTATGGACCGCCCCCTGCAGGAAGAGGAGCCCCCGCCACCCCCAC  
CGTTCACCTCCTACATCGTGTCCACCCCTCCTGGAGGCTTTCCCCCTCC  
CCAGGGCTTCCCTCAGGGCTACGGTGCCCCGCCACAGTTCAGTTTTGGC  
TACGGGCTCCACCTCCACCGCCAGATCAGTTTGCCCTCCGGGGGTTTC  
CTCCTCCACCAGCCACTCCCGGGGACGACCTCTGGCTTTCCACCGCC  
TCCGTCTCAGGCTGCCCCGGACATGAGCAAGCCCCGACAGCTCAGCCA  
GACTTCCCTATGGTCAGTATGCAGGTTACGGGCAGGACTTGAGTGGCT  
TCGGACAGGGCTTCTCAGACCCAGCCAGCAGCCTCCTTCCCTACGGGGG  
TCCCTCCGTGCCAGGGTCGGGGGGCCCCCGCCGGCGGCAGCGGCTTT  
GGACGAGGGCAGAACCACAACGTGCAAGGGTTCCACCCCTACCGACGCT  
agggatcc