

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of AKAP1 [1 – 903]

Enzyme description:- AKAP1 [1 – 903]

Clone number:- DU 15767

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 124, 071.58 daltons

Average Mass 124, 149.76 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.97

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

Division of Signal Transduction Therapy

Clone Data Sheet

AKAP1 [1 – 903]

<u>Protein</u>	AKAP1 [1 – 903]
<u>Clone number</u>	DU 15767
<u>Species</u>	Human
<u>Accession number</u>	Q92667
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGPLGSMAIQFRSLFPLALP GMLALLGWWFFSRKKGHVSSHDEQQVEAGAVQLRADPAIKEPLPVEDV CPKVSTPPSVTEPPEKELSTVSKLPAEPPALLQTHPPCRRSESSGILP NTTDMRLRPGTRRDDSTKLELALTGGEAKSIPLECPLSAPKGVLFSSKS AEVCKQDSPFSRVPRKVQPGYPVPAEKRSSGERARETGGAEGTGDAVL GEKVLEEALLSREHVLELENSKGPSLASLEGEEDKGKSSSSQVVGVPVQE EEYVAEKLPSRFIESAHTELAKDDAAPAPPVADAKAQDRGVEGELGNEE SLDRNEEGLDRNEEGLDRNEESLDRNEEGLDRNEEIKRAAFQIISQVIS EATEQVLATTVGKVAGRVCAQSQLOGQKEESCVPVHQKTVLGPDTAEPA TAEAAVAPPDAGLPLPGLPAEGSPPKTYVSCLSLLSSPTKDSKPNIS AHHISLASCLALTPSEELPDRAIGLVEDATCVTCMSDSSQSVPLVASP GHCSDFSSTSGLEDSTETSSSPRDKAITPPLPESTVPFSSNGVLKGELS DLGAEDGWTMDAEADHSGGSDRNSMDSVDSCCSLKKTESFQNAQAGSNP KKVDLI IWEIEVPKHLVGRLIGKQGRYVSFLKQTS GAKIYISTLPYTQS VQICHIEGSQHHVDKALNLIGKKFKELNLNIIYAPPLPSLALPSLPMTS WLMLPDGITVEVIVVNQVNAGHLFVQQHPTPTFHALRSLDQQMYLCYSQ PGIPTLPTPVEITVICAAPGADGAWWRAQVVASYEETNEVEIRYVDYGG YKRVKVDVLRQIRSDFTLPFQGAEVLLDSVMPLSDDQFSPEADAAMS EMTGNTALLAQVTSYSPTGLPLIQLWSVVGDEVVLINRSLVERGLAQWV DSYYTSL
<u>Native sequence</u>	Amino acids M1 – L903 (end) of human AKAP1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Division of Signal Transduction Therapy

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*I sites of pGEX6P1

Nucleotide Sequence of Insert:

```
ggatccATGGCAATCCAGTTCGGTTCGCTCTTCCCCTTGGCATTGCCTGGGATGCTGGCGCTCCTC
GGCTGGTGGTGGTTTTTCTCTCGTAAAAAAGGCCATGTCAGCAGCCATGATGAGCAGCAGGTGGAG
GCTGGTGTGTGCAGCTGAGGGCTGACCTGCCATCAAGGAACCTCTCCCCGTGGAAGACGTCTGT
CCCAAAGTAGTGTCCACACCCCCCAGTGTACAGAGCCTCCAGAAAAGGAAGTGTCCACCGTGAGC
AAGCTGCCTGCAGAGCCCCCAGCATTGCTCCAGACACACCCACCTTGCCGAAGATCAGAGTCCTCG
GGCATTCTTCCCTAACACCACAGACATGAGATTGCGACCAGGAACACGCAGAGATGACAGTACAAAG
CTGGAGCTAGCCCTGACAGGTGGTGAAGCCAAATCGATTCTCTAGAGTGCCCCCTTTCAGCCCCA
AAGGGTGTAATCTCTCCAGCAAATCAGCTGAGGTGTGTAAGCAAGATTCCCCCTTCAGCAGGGTG
CCAAGGAAGGTCCAGCCAGGCTACCCCGTAGTCCCCGCAGAGAAGCGTAGCTCTGGGGAGAGGGCA
AGAGAGACAGGTGGGGCCGAAGGGACTGGTGTATGCCGTGTTGGGGGAAAAGGTGCTTGAAGAAGCT
CTGTTGTCTCGGGAGCATGTCTTGGAATTGGAGAACAGCAAGGGCCCCAGCCTGGCCTCTTTAGAG
GGGGAAGAAGATAAGGGGAAGAGCAGCTCATCCCAGGTGGTGGGGCCAGTGCAGGAGGAAGAGTAT
GTAGCAGAGAAGTTGCCAAGTAGGTTTCATCGAGTCGGCTCACACAGAGCTGGCAAAGGACGATGCG
GCGCCAGCACCCCCAGTCGCAGACGCCAAAGCCCAGGATAGAGGTGTGAGGGGAGAACTGGGCAAT
GAGGAGAGCTTGGATAGAAATGAGGAGGGCTTGGATAGAAATGAGGAGGGCTTGGATAGAAATGAG
GAGAGCTTGGATAGAAATGAGGAGGGCTTGGATAGAAATGAGGAGATTAAGCGGGCTGCCTTCCAG
ATAATCTCCCAAGTGATCTCAGAAGCAACCGAACAGGTGCTGGCCACCACGGTTGGCAAGGTGCA
GGTCGTGTGTGTCAGGCCAGTCAGCTCCAAGGGCAGAAGGAAGAGAGCTGTGTCCAGTTTACCAG
AAAAGTGTCTTGGGCCCAGACACTGCGGAGCCTGCCACAGCAGAGGCAGCTGTTGCCCCGCCGGAT
GCTGGCCTCCCCCTTGCCAGGCCTACCAGCAGAGGGCTCACCACCACCAAAGACCTACGTGAGCTGC
CTGAAGAGCCTTCTGTCCAGCCCCACCAAGGACAGTAAGCCAAATATCTCTGCACACCACATCTCC
CTGGCCTCCTGCCTGGCACTGACCACCCCCAGTGAAGAGTTGCCGGACCGGGCAGGCATCCTGGTG
GAAGATGCCACCTGTGTACCTGCATGTCAGACAGCAGCCAAAGTGTCCCTTTGGTGGCTTCTCCA
GGACACTGCTCAGATTCTTTTACGACTTCAGGGCTTGAAGACTCTTGCACAGAGACCAGCTCGAGC
CCCAGGGACAAGGCCATCACCCCGCCACTGCCAGAAAAGTACTGTGCCCTTCAGCAATGGGGTGCTG
AAGGGGGAGTTGTGCACTTGGGGGCTGAGGATGGATGGACCATGGATGCGGAAGCAGATCATTTCA
GGAGGTCTGACAGGAACAGCATGGATTCCGTGGATAGCTGTTGCAGTCTCAAGAAGACTGAGAGC
TTCCAAAATGCCCAGGCAGGCTCCAACCCTAAGAAGGTCGACCTCATCATCTGGGAGATCGAGGTG
CCAAAGCACTTAGTTCGGTTCGGCTAATTGGCAAGCAGGGGGCGCTATGTGAGTTTTCTGAAGCAAACA
TCTGGTGCCAAGATCTACATTTCAACCCTGCCTTACACCCAGAGCGTCCAGATCTGCCACATAGAA
GGCTCTCAACATCATGTAGACAAAGCGCTGAACTTGATTGGTAAGAAGTTCAAAGAGCTGAACCTC
ACCAATATCTACGCTCCCCCATTTGCCTTCACTGGCACTGCCTTCTCTGCCGATGACATCCTGGCTC
ATGCTGCCTGATGGCATCACCGTGGAGGTCAATGTTGGTCAACCAGGTCAATGCCGGGCACCTGTTT
GTGCAGCAGCACACACACCCCTACCTTCCACGCGCTGCGCAGCCTCGACCAGCAGATGTACCTCTGT
TACTCTCAGCCTGGAATCCCCACCTTGCCCACCCCAGTGGAAATAACGGTCATCTGTGCCGCCCCCT
GGTGGCGACGGGGCCTGGTGGCGAGCCCAAGTGGTTGCCTCCTACGAGGAGACCAACGAAGTGGAG
ATTCGATACGTGGACTACGGCGGATATAAGAGGGTGAAAGTAGACGTGCTCCGGCAAATCAGGTCT
GACTTTGTACCCCTGCCGTTTTCAGGGAGCAGAAGTCCCTTCTGGACAGTGTGATGCCCTGTGACAG
GATGACCAGTTTTTACCAGGAAGCAGATGCCGCCATGAGCGAGATGACGGGGAATACAGCACTGCTT
GCTCAGGTGACAAGTTACAGTCCAACCTGGTCTTCTCTGATTACAGCTGTGGAGTGTGGTTGGAGAT
GAAGTGGTGTGATAAACCAGGTCCCTGGTGGAGCGAGGCCTTGCCCAGTGGGTAGACAGCTACTAC
ACAAGCCTTtgagcggccgc
```