Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Annexin A2 [2 – 339]

Enzyme description:- Annexin A2 [2 - 339]

Clone number:- DU 600

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and HA

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 66, 613.96 daltons
Average Mass 66, 656.49 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.04

Purity:- >80 %

Activation protocol:- Constitutively active

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

Assay:- Substrate for PKC

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Clone Data Sheet

Annexin A2 [2 – 339]

Protein	Annexin A2 [2 - 339]
Clone number	DU 600
Species	Human
Accession number	NM_004039.2
Tags	N-terminal GST and HA
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSAT*MYPYDVPDYA***ST** **VHEILCKLSLEGDHSTPPSAYGSVKAYTNFDAERDALNIETAIKTKGVD** **EVTIVNILTNRSNAQRQDIAFAYQRRTKKELASALKSALSGHLETVILG** **LLKTPAQYDASELKASMKGLGTDEDSLIEIICSRTNQELQEINRVYKEM** **YKTDLEKDIISDTSGDFRKLMVALAKGRRAEDGSVIDYELIDQDARDLY** **DAGVKRKGTDVPKWISIMTERSVPHLQKVFDRYKSYSPYDMLESIRKEV** **KGDLENAFLNLVQCIQNKPLYFADRLYDSMKGKGTRDKVLIRIMASRSE** **VDMLKIRSEFKRKYGKSLYYYIQQDTKGDYQKALLYLCGGDD**
Native sequence	Amino acids S2– D339 (end) of human Annexin A2. Residue S244of the fusion protein is equivalent to S2 of the native enzyme. The GST tag is located at residues 1 – 220 and the HA tag is located at residues 235 - 243.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 229
Cloning sites	*Bam*H1 and *Sal*1sites of pGEX 6P-1

Nucleotide Sequence of insert

ggatccgccaccatgtacccatacgatgtgccagattacgccTCTACTG
TTCACGAAATCCTGTGCAAGCTCAGCTTGGAGGGTGATCACTCTACACC
CCCAAGTGCATATGGGTCTGTCAAAGCCTATACTAACTTTGATGCTGAG
CGGGATGCTTTGAACATTGAAACAGCCATCAAGACCAAAGGTGTGGATG
AGGTCACCATTGTCAACATTTTGACCAACCGCAGCAATGCACAGAGACA
GGATATTGCCTTCGCCTACCAGAGAAGGACCAAAAAGGAACTTGCATCA
GCACTGAAGTCAGCCTTATCTGGCCACCTGGAGACGGTGATTTTGGGCC
TATTGAAGACACCTGCTCAGTATGACGCTTCTGAGCTAAAAGCTTCCAT
GAAGGGGCTGGGAACCGACGAGGACTCTCTCATTGAGATCATCTGCTCC
AGAACCAACCAGGAGCTGCAGGAAATTAACAGAGTCTACAAGGAAATGT
ACAAGACTGATCTGGAGAAGGACATTATTTCGGACACATCTGGTGACTT
CCGCAAGCTGATGGTTGCCCTGGCAAAGGGTAGAAGAGCAGAGGATGGC
TCTGTCATTGATTATGAACTGATTGACCAAGATGCTCGGGATCTCTATG
ACGCTGGAGTGAAGAGGAAAGGAACTGATGTTCCCAAGTGGATCAGCAT
CATGACCGAGCGGAGCGTGCCCCACCTCCAGAAAGTATTTGATAGGTAC
AAGAGTTACAGCCCTTATGACATGTTGGAAAGCATCAGGAAAGAGGTTA
AAGGAGACCTGGAAAATGCTTTCCTGAACCTGGTTCAGTGCATTCAGAA
CAAGCCCCTGTATTTTGCTGATCGGCTGTATGACTCCATGAAGGGCAAG
GGGACGCGAGATAAGGTCCTGATCAGAATCATGGCCTCCCGCAGTGAAG
TGGACATGTTGAAAATTAGGTCTGAATTCAAGAGAAAGTACGGCAAGTC
CCTGTACTATTATATCCAGCAAGACACTAAGGGCGACTACCAGAAAGCG
CTGCTGTACCTGTGTGGTGGAGATGACtgagtcgac