

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PKB alpha PH domain [1 - 149]

Protein description:- PKB alpha PH domain [1 - 149]

Clone number:- DU 1537

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 4 mg/L

Calculated molecular mass:- 44, 129 daltons

Purity:- 95 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -20 °C

Division of Signal Transduction Therapy

CLONE DATA SHEET

PKB alpha PH domain [1 - 149]

<u>Protein</u>	PKB alpha PH domain [1 - 149]
<u>Clone number</u>	DU 1537
<u>Species</u>	Human
<u>Accession no</u>	M63167
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWR NKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGG CPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKL PEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPM CLDAFPKLVCFKKRIEAIIPQIDKYLKSSKYIAWPLQGWQATF GGGDHPPKSDLVPRGSPEFMSDVAIVKEGWLHKRGEYIKTWR PRYFLLKNDGTFIGYKERPQDVDQREAPLNNFSVAQCQLMKT ERPRPNTFIIIRCLQWTTVIERTFHVETPEEREWTTAIQTVA DGLKKQEEEEEMDFRSGSPSDNSGAEEMEVS LAKPKHRVTMNE
<u>Native sequence</u>	Amino acids M1 – E149 of human PKB alpha. [Full length protein ends at residue A480] Residue M230 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 - 220
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) residues 221 - 226
<u>Cloning sites</u>	<i>Eco</i> R1 of pGEX-4T-1
<u>Nucleotide sequence of insert</u>	GAATTCATGAGCGACGTGGCTATTGTGAAGGAGGGTTGGCTG CACAAACGAGGGAGTACATCAAGACCTGGCGGCCACGCTAC TTCTCCTCAAGAATGATGGCACCTTCATTGGCTACAAGGAG CGGCCGCAGGATGTGGACCAACGTGAGGCTCCCTCAACAAC TTCTCTGTGGCGCAGTGCCAGCTGATGAAGACGGAGCGGCC CGGCCAACACCTTCATCATCCGCTGCCTGCAGTGGACCA CTGTCATCGAACGCACCTTCCATGTGGAGACTCCTGAGGAGC GGGAGGAGTGGACAACCGCCATCCAGACTGTGGCTGACGGCC TCAAGAAGCAGGAGGAGGAGATGGACTTCCGGTTCGGGCT CACCCAGTGACAACTCAGGGGCTGAAGAGATGGAGGTGTCCC TGGCCAAGCCCAAGCACCGCGTGACCATGAACGAGtga

Division of Signal Transduction Therapy