Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of PKB alpha PH domain [1 - 123]

Protein description:- PKB alpha PH domain [1 - 123]

Clone number:- DU 1789

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 4 mg/L

Calculated molecular mass:- 41, 462 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

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CLONE DATA SHEET

PKB alpha PH domain [1 - 123]

Protein	PKB alpha PH domain [1 - 123]
Clone number	DU 1789
Species	Human
Accession no	M63167
Tags	N-terminal GST
Bacterially expressed prtoein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWR NKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGG CPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKL PEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPM CLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATF GGGDHPPKSDLEVLFQGPLGS**MSDVAIVKEGWLHKRGEYIKT** **WRPRYFLLKNDGTFIGYKERPQDVDQREAPLNNFSVAQCQLM** **KTERPRPNTFIIRCLQWTTVIERTFHVETPEEREEWTTAIQT** **VADGLKKQEEEEMDFRSG**
Native sequence	Amino acids M1 – G123 of human PKB alpha. [Full length protein ends at residue A480] Residue M232 of the fusion protein is equivalent to M1 of the native protein. The GST tag is located at residues 1 - 220
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 of pGEX-6P-1
Nucleotide sequence of insert	GGATCCATGAGCGACGTGGCTATTGTGAAGGAGGGTTGGCTG CACAAACGAGGGGAGTACATCAAGACCTGGCGGCCACGCTAC TTCCTCCTCAAGAATGATGGCACCTTCATTGGCTACAAGGAG CGGCCGCAGGATGTGGACCAACGTGAGGCTCCCCTCAACAAC TTCTCTGTGGCGCAGTGCCAGCTGATGAAGACGGAGCGGCCC CGGCCCAACACCTTCATCATCCGCTGCCTGCAGTGGACCACT GTCATCGAACGCACCTTCCATGTGGAGACTCCTGAGGAGCGG GAGGAGTGGACAACCGCCATCCAGACTGTGGCTGACGGCCTC AAGAAGCAGGAGGAGGAGGAGATGGACTTCCGGTCGGGCtga ggatcc

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