Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of IkappaB alpha S32A S36A [2 - 54]

Enzyme description:-   IkappaB alpha S32A S36A [2 – 54]

Clone number:-   DU 1445

Source:-   Recombinant

Expression system:-   *E.coli*

Tag:-   N-terminal GST

Purification method:-   GSH Sepharose

Calculated molecular mass:-
Monoisotopic   33, 118.72 daltons
Average Mass   33, 140.22 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:-   5.24

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

IkappaB alpha S32A S36A [2 – 54]

Protein	IkappaB alpha S32A S36A [2 – 54]
Clone number	DU 1445
Species	Human
Accession number	BC004983
Tags	N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**FQAAERPQEWAMEG
PRDGLKKERLLDDRHDAGLDAMKDEEYEQMVKELQEIRL**

Native sequence

Amino acids F2 – L54 (end residue is L317) of human IkappaB alpha. Residue F232 of the fusion protein is equivalent to F2 of the native enzyme. The GST tag is located at residues 1 – 220. The protein has an S32A and an S36A mutation. Residue S32 is equivalent to residue A262 of the fusion protein. Residue S36 is equivalent to residue A266 of the fusion protein.

Protease cleavage

PreScission (LEVLFQGP) residues 221 - 228

Cloning sites

*Bam*H1 and *Eco*R1 sites of pGEX6P-1

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Nucleotide Sequence Of Insert

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ggatccTTCCAGGCGGCCGAGCGCCCCCAGGAGTGGGCCATGGAGGGCC
CCCGCGACGGGCTGAAGAAGGAGCGGCTACTGGACGACCGCCACGACgc
CGGCCTGGACgcCATGAAAGACGAGGAGTACGAGCAGATGGTCAAGGAG
CTGCAGGAGATCCGCCTCtgagaattc
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