

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of IkappaB alpha [2 - 54]**

**Enzyme description:-** IkappaB alpha [2 – 54]

**Clone number:-** DU 1442

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 33, 150.71 daltons

Average Mass 33, 172.22 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 [2 – 54]**

|   |   |
|---|---|
| <b><u>Protein</u></b>                       | IkappaB alpha [2 – 54]  |
| <b><u>Clone number</u></b>                  | DU 1442   |
| <b><u>Species</u></b>                       | Human   |
| <b><u>Accession number</u></b>              | BC004983  |
| <b><u>Tags</u></b>                          | N-terminal GST  |
| <b><u>Bacterially expressed protein</u></b> | <p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG<br/>LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA<br/>VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH<br/>VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS<br/>KYIAWPLQGWQATFGGGDHPKSDLEVL FQGPLGS<b>FQAAERPQEWAMEG</b><br/><b>PRDGLKKERLLDDRHDSGLDSMKDEEYEQMVKELQEIRL</b></p> |
| <b><u>Native sequence</u></b>               | <p>Amino acids F2 – L54 (end residue is L317) of human IkappaB alpha.<br/>Residue F232 of the fusion protein is equivalent to F2 of the native<br/>enzyme. The GST tag is located at residues 1 – 220.</p>  |
| <b><u>Protease cleavage</u></b>             | PreScission ( <u>LEVLFQGP</u> ) residues 221 - 228  |
| <b><u>Cloning sites</u></b>                 | <i>Bam</i> H1 and <i>Eco</i> R1 sites of pGEX6P-1   |

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Nucleotide  
Sequence Of  
Insert

ggatccTTCCAGGCGGCCGAGCGCCCCAGGAGTGGGCCATGGAGGGCC  
CCCGCGACGGGCTGAAGAAGGAGCGGCTACTGGACGACCGCCACGACAG  
CGGCCTGGACTCCATGAAAGACGAGGAGTACGAGCAGATGGTCAAGGAG  
CTGCAGGAGATCCGCCTctgagaattc