

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of Alpha Synuclein [1 - 140]**

**Enzyme description:-** Alpha Synuclein [1 – 140]

**Clone number:-** DU 30005

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 41, 257.86 daltons

Average Mass 41, 284.32 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.13

**Purity:-** >80 %

**Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,  
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

**Storage temperature:-** -70 °C

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### **Clone Data Sheet**

#### **Alpha Synuclein [1 - 140]**

<b><u>Protein</u></b>	Alpha Synuclein [1 - 140]
<b><u>Clone number</u></b>	DU 30005
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_001146054.1
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSMDFVMKGLS <b>KAKEGVVAAA</b>EKTQGVAA<b>EAGKTKEGVLYVGSKTKEGVVHGVATVAE</b> <b>KTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEE</b> <b>GAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids M1 – A140 (end) of human alpha synuclein. Residue M232 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<b><u>Protease cleavage</u></b>	Prescission site ( <u>LEVLFOGP</u> ) at residues 221 – 228
<b><u>Cloning sites</u></b>	<i>Bgl</i> 2 ( <i>Bam</i> H1 site) and <i>Not</i> 1 site of pGex6P-1
<b><u>Nucleotide sequence of insert</u></b>	<p>agatctATGGATGTATTCATGAAAGGACTTTCAAAGGCCAAGGAGGGA GTTGTGGCTGCTGCTGAGAAAACCAACAGGGTGTGGCAGAAGCAGCA GGAAAGACAAAAGAGGGTGTCTCTATGTAGGCTCCAAAACCAAGGAG GGAGTGGTGCATGGTGTGGCAACAGTGGCTGAGAAGACCAAAGAGCAA GTGACAAATGTTGGAGGAGCAGTGGTGACGGGTGTGACAGCAGTAGCC CAGAAGACAGTGGAGGGAGCAGGAGCATTGCAGCAGCCACTGGCTTT GTCAAAAAGGACCAGTTGGGCAAGAATGAAGAAGGAGCCCCACAGGAA GGAATTCTGGAAGATATGCCTGTGGATCCTGACAATGAGGCTTATGAA ATGCCTTCTGAGGAAGGGTATCAAGACTACGAACCTGAAGCCTaagcg gccgc</p>