

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of KLHL3 [290 – 587]**

**Enzyme description:-** KLHL3 [290 – 5587]

**Clone number:-** DU 44387

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 59, 134.62 daltons

Average Mass 59, 172.75 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.35

**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

#### KLHL3 [290 – 587]

<b><u>Protein</u></b>	KLHL3 [290 – 587]
<b><u>Clone number</u></b>	DU 44387
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_017415.2
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSMRTKPRTPVSLPKV <b>MI VVGQAPKAIRSVECYDFEEDRWDQIAELPSRRCRAGVVF MAGHVYA</b> <b>VGGFNGSLRVRTVDVYDGVKDQWTSIASMQERRSTLGA AVLNDLLYAVG</b> <b>GFDGSTGLASVEAYS YKTNEWFFVAPMNTRRSSVGVGVVEGKLYAVGGY</b> <b>DGASRQCLSTVEQYNPATNEWIYVADMSTRRS GAGVGVLSGQLYATGGH</b> <b>DGPLVRKSVEVYDPGTNTWKQVADMNMCR RNAGVCAVNGLLYVVGDDG</b> <b>SCNLASVEYYNPVTDKWTLLPTNMSTGRSYAGVAVIHKSL</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids R290 – L587 (end) of human KLHL3. Residue R233 of the fusion protein is equivalent to R290 of the native 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>Bgl</i> 1 and <i>Not</i> 1 sites into <i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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### **Nucleotide Sequence of Insert:**

agatctatgAGGACCAAGCCCAGGACTCCAGTCAGCCTTCCCAAGGTCATGATTGTGGTTGGCGGC  
CAGGCACCCAAGGCAATCCGCAGTGTGGAGTGCTATGATTTTCGAGGAGGACCGGTGGGATCAGATT  
GCTGAGCTTCCTTCCAGAAGATGCAGAGCAGGTGTGGTGTTCATGGCTGGCCACGTGTATGCCGTG  
GGAGGGTTTAATGGCTCACTGCGGGTGCGGACAGTGGATGTGTATGACGGCGTGAAGGACCAGTGG  
ACGTCCATTGCCAGCATGCAGGAGCGCCGGAGCACACTGGGCGCAGCGGTGCTCAATGACTTGCTC  
TACGCAGTGGGAGGCTTTGATGGCAGTACTGGCCTAGCATCGGTGGAAGCCTACAGCTACAAGACC  
AACGAGTGGTTCTTTGTGGCCCCGATGAACACGCGGCGGAGCAGTGTGGGTGTGGGCGTTGTGGAG  
GGGAAGCTATATGCTGTTGGGGTTATGATGGAGCTTCCCGCCAGTGTCTGAGCACTGTGGAGCAG  
TACAACCCAGCGACCAATGAATGGATATACGTGGCGGACATGAGCACCCGCCGAGTGGCGCAGGG  
GTTGGAGTGCTTAGCGGACAGCTGTACGCCACAGGTGGGCATGATGGGCCTTTGGTGAGGAAGAGC  
GTTGAGGTTTACGATCCTGGAACAAATACCTGGAAGCAAGTGGCAGACATGAACATGTGCCGGCGC  
AACGCAGGGGTCTGTGCAGTAAATGGGCTCCTGTATGTGGTTGGAGGGGATGATGGATCCTGCAAC  
TTGGCTTCGGTGGAGTACTACAATCCTGTCACTGACAAATGGACGCTGCTTCCAACGAACATGAGC  
ACGGGGCGGAGCTATGCAGGTGTTGCCGTGATTCACAAGTCCTTGtgagcggccgc

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