

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of PRAS40 [1 - 256]**

**Enzyme description:-** PRAS40 [1 - 256]

**Clone number:-** DU 5458

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 54, 199.13 daltons

Average Mass 54, 233.54 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.00

**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**

#### **PRAS40 [1 - 256]**

<b><u>Protein</u></b>	PRAS40 [1 - 256]
<b><u>Clone number</u></b>	DU 5458
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_001098633.3
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSMASGRPEELWEAVV <b>GAAERFRARTGTELVLLTAAPPPPPRPGPCAYPAHGRGALAEAARRCLH</b> <b>DIALAHRAATAARPPAPPAPQPPSPTPSPPRPTLAREDNEEDEDEPTE</b> <b>TETSQEQLGISDNGGLFVMEDEATLQDLPPFCESDPESTDDGSLSEETP</b> <b>AGPPTCSVPPASALPTQQYAKSLPVSVPVWGFKEKRTEARSSDEENGPP</b> <b>SSPDLDRIAASMRALVLR EAEDTQVFGDLPRPRLNTSDFQKLKRKY</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids M1 – Y256 (end) of human PRAS40. Residue M232 of the 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 ( <u>LEVLFQGP</u> ) residues 221 - 228
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 of pGEX6P-3

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**Nucleotide**  
**Sequence of insert**

ggatccATGGCGTCGGGGCGCCCCGAGGAGCTGTGGGAGGCCGTGGTGG  
GGGCCGCCGAGCGCTTCCGGGCCCGGACTGGCACGGAGCTGGTGCTGCT  
GACCGCGGCCCCGCCGCCACCACCCCGCCGGGCCCTGTGCCTATCCT  
GCCCATGGTTCGAGGAGCCCTGGCGGAGGCAGCGCGCCGTTGCCTCCACG  
ACATCGCACTGGCCACAGGGCTGCCACTGCTGCTCGGCCTCCTGCGCC  
CCCACCAGCACACAGCCACCCAGTCCCACACCCAGCCCACCCCGGCCT  
ACCCTGGCCAGAGAGGACAACGAGGAGGACGAGGATGAGCCCACAGAGA  
CAGAGACCTCCGGGGAGCAGCTGGGCATTAGTGATAATGGAGGGCTCTT  
TGTGATGGATGAGGACGCCACCCCTCCAGGACCTTCCCCCTTCTGTGAG  
TCAGACCCCGAGAGTACAGATGATGGCAGCCTGAGCGAGGAGACCCCG  
CCGGCCCCCCCCACCTGCTCAGTGCCCCCAGCCTCAGCCCTACCCACACA  
GCAGTACGCCAAGTCCCTGCCTGTGTCTGTGCCCGTCTGGGGCTTCAAG  
GAGAAGAGGACAGAGGCGCGGTCATCAGATGAGGAGAATGGGCCGCCCT  
CTTCGCCCCGACCTGGACCGCATCGCGGCGAGCATGCGCGCGCTGGTGCT  
GCGAGAGGCCGAGGACACCCAGGTCTTCGGGGACCTGCCACGGCCGCGG  
CTTAACACCAGCGACTTCCAGAAGCTGAAGCGGAAATATtgaggatcc