

***MRCPPU Reagents and Services***

**Standard Operating Procedure**

**Preparation of CDKL5 [350 – 650]**

**Enzyme description:-** CDKL5 [350 – 650]

**Clone number:-** DU 50406

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Agarose

**Calculated molecular mass:-**

Monoisotopic 59, 590.85 daltons

Average Mass 59, 628.20 daltons

[cysteines reduced, methionines have not been oxidised

**Theoretical pI:-** 8.75

**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, 1 mM benzamidine, 0.2 mM PMSF

**Storage temperature:-** -70 °C

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### Clone Data Sheet

#### CDKL5 [350 - 650]

<b><u>Protein</u></b>	CDKL5 [350 - 650]
<b><u>Clone number</u></b>	DU 50406
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_003159.3
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	MSPILGYWKIKGLVQPTRLLEKYEEHYERDEGDKWRNKKFELG LEFPNLPLYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGA VLDIYGVSRIAYSKDFETLKVDFLSKLPEMPLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDELVLFQGPLGS <b>SVGLPRADEGLPAN</b> <b>ESFLNGNLAGASLSPHTKTYQASSQPGSTS</b> KDLTNNNIPHLLSPKEAK SKTEFDENIDPKPSEGPGTKYLKSNSRSQQNRHSFMESSQSKAGTLQPN EKQSRHSYIDTIPQSSRSPSYRTKAKSHGALSDSKSVSNLSEARAQIAE PSTSRYFPSSCLDLSPTSPTRHSDTRTLLSPSGRNNRNEGTLDSRR TTTRHSKTMEELKLPEHMDSSHSHSLSAPHEFSYGLGYTSPFSSQQRPHRHSMYVTRDKVRAKGGLDGSLSIGQGMAARANSIQLLSPQPG
<b><u>Native sequence</u></b>	Amino acids S350 – G650 (K1030 end residue) of human CDKL5. Residue S232 of the fusion protein is equivalent to S350 of the native enzyme. The GST tag is located at residues 1 – 220.
<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>Not</i> 1 of pGEX6P-1