

MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of CDKL5 [300 – 600 mouse]

Enzyme description:- CDKL5 [300 – 600 mouse]

Clone number:- DU 53266

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-

Monoisotopic 59, 973.04 daltons

Average Mass 60, 010.47 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.92

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 [300 – 600 mouse]

<u>Protein</u>	CDKL5 [300 – 600 mouse]
<u>Clone number</u>	DU 53266
<u>Species</u>	Mouse
<u>Accession number</u>	NM_001024624.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL<u>FQGP</u>LG<u>SRLLDRSPSRSTKR</u> <u>KPYHVESSTLSNRNQSTKGAALQTHHRSNSKDIQNLSVGLPRAEEGLPA</u> <u>NESFLNGNLAGATLSPMHTKTYQASTQPGSSSKDLTNNNI PHLLSPKEA</u> <u>KSKTEFDENIDTKPSEGPGTKYLKSSTRSQQNRHSFMESSQSKAGTLQP</u> <u>SEKQSRHSYIDTI PQSSRSPSYRTKAKSHGALSDSKSVSNLSEARAQIT</u> <u>ETNTSRYFPSSCLDLNSPTSPTPTRHTDTRTLLSPSGRNNRNEGTLDSR</u> <u>RTTTRHSKTMEELKLP EHMDSHSHSLSAPHESFSYGLGYTS</u></p>
<u>Native sequence</u>	Amino acids Q300 – S600 (Q938 end residue) of mouse CDKL5. Residue Q232 of the fusion protein is equivalent to Q300 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (<u>LEVL<u>FQGP</u></u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 of pGEX6P-1