

Certificate of Analysis

CDKL1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 16-002, 16-002-K, 16-002M

Parent Lot # D17EP002N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, human CDKL1 amino acids 1-303 expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 92% by SDS-PAGE and Coomassie blue staining. MW = 39kDa.

Specific Activity (Parent lot# D17EP002N): 13U/mg, where one unit of CDKL1 activity is defined as 1nmol phosphate incorporated into 250 μ M RRRFRPASPLRGPPK per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 1.36mg/ml of enzyme in 50mM Tris/HCl pH7.5, 280mM NaCl, 260mM imidazole, 0.09mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.9mM benzamidine, 0.2mM PMSF, 0.09% 2-mercaptoethanol. Frozen solution.

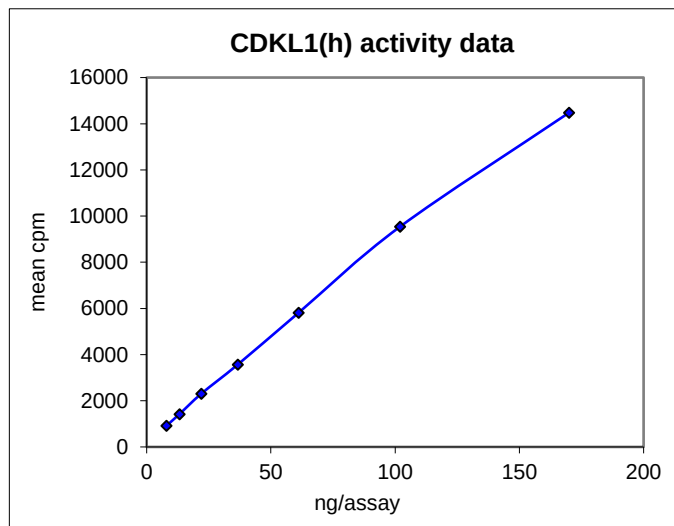
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

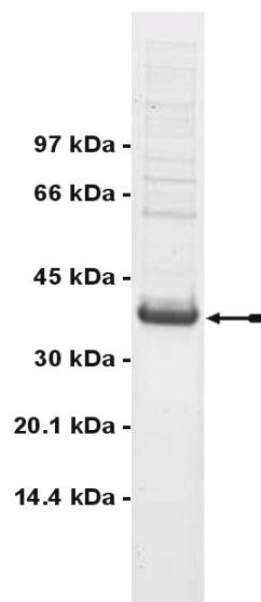
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 7.9–170ng of this lot of enzyme phosphorylated 250 μ M RRRFRPASPLRGPPK in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as CDKL1 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of CDKL1, active.

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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. RRRFRPASPLRGPPK:** Use at a final assay concentration of 250µM. Prepare a 25mM stock and add 2.5µl of stock per assay point.
- 3. CDKL1:, active** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7.9–170ng per assay point.
- 4. [γ-³³P]ATP:** 2.5 x MgAc/[γ³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of RRRFRPASPLRGPPK.
3. Add **2.5µl (7.9–170ng) CDKL1, active.**
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ-³³P]ATP mixture.
6. Incubate for 30 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the dried filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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CDKL1, active Sequence Information

<u>Protein</u>	Human CDKL1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M32 of the recombinant protein is equivalent to M1 of human CDKL1
<u>Accession number</u>	GenBank NM_004196.3

Recombinant CDKL1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF SMMEKYEKIG KIGEGSYGVV FKCRNRDTGQ
61 IVAIKKFLES EDDPVIKKIA LREIRMLKQL KHPNLVNLLE VFRRKRRLHL VFEYCDHTVL
121 HELDRYQRGV PEHLVKSITW QTLQAVNFCH KHNCIHRDVK PENILITKHS VIKLCDFGFA
181 RLLTGPSDYY TDYVATRWR YR SPELLVGDTQ YGPPVDVWAI GCVFAELL SG VPLWPGKSDV
241 DQLYLIRKTL GDLIPRHQQV FSTNQYFSGV KIPDPEDMEP LELKFPNISY PALGLLKGCL
301 HMDPTQRLTC EQLLHHPYFE NIREIEDLAK EHNK

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Recombinant CDKL1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc tctatgatgg agaagtatga aaaaattggg
121 aaaattggag aaggatccta tggagttggt ttcaaatgta gaaacagggg cacgggtcag
181 attgtggcca tcaagaagtt tctggaatca gaagatgacc ctgtcataaa gaaaattgcc
241 cttcgggaaa tccgaatgct caagcaactc aagcatccca accttggtta cctcctggaa
301 gtcttcagga ggaaacggag gcttcacctg gtgtttgaat attgtgacca cacagttctc
361 catgagttgg acagatacca aagaggggta ccagaacatc tcgtgaagag cataacttgg
421 cagacactgc aagctgtaaa tttttgccat aaacacaatt gcatacatag agacgtgaag
481 ccagaaaata tcctcatcac gaaacattcc gtgattaagc tttgtgactt tggatttgct
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961 aacatcagag aaatagagga tttggcaaaa gaacacaaca aataa

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