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Product Information

CDC25C, active, GST tagged, human recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5007**
Storage Temperature –70 °C

Synonym: CDC25

Product Description
CDC25C (also known as cell division cycle 25 homolog C) is a member of the CDC25 phosphatase family. CDC25C is highly conserved during evolution and it plays a key role in the regulation of cell division. CDC25C activates the partially purified p34 (cdc2)/ cyclin B complex, directs the dephosphorylation of cyclin B-bound CDC2, and triggers entry into mitosis.[1] CDC25C also suppresses p53-induced growth arrest. The regulation of CDC25B phosphorylation by p38 is a critical event for initiating the G_2/M checkpoint after ultraviolet radiation.[2]

Recombinant, full-length, human CDC25C was expressed in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is BC019089. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, 25% glycerol.

Molecular mass: ~84 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 19–25 nmol/min/mg (see Figure 2)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

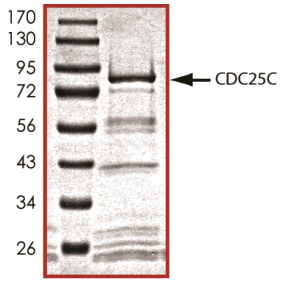
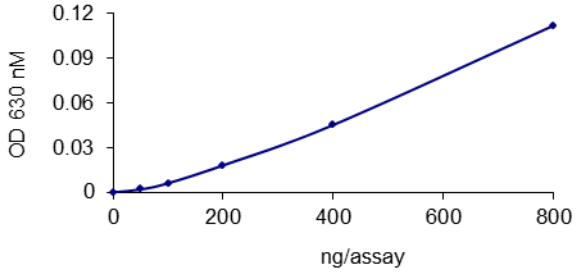


Figure 2.
Specific Activity of Typical Lot
19–25 nmol/min/mg



Procedure

Preparation Instructions

Phosphatase Dilution Buffer – 100 mM Tris-HCl, pH 8.2, 40 mM NaCl, 1 mM DTT, and 20% glycerol.

Phosphatase Solution – Dilute the active CDC25C (0.1 μg/μl) with Phosphatase Dilution Buffer to the desired concentration.

Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active CDC25C for optimal results.

Substrate Assay Solution – OMFP (3-O-methyl-fluorescein phosphate) diluted in Phosphatase Dilution Buffer to a final concentration of 500 μM.

Detection Solution – BIOMOL Green™ Reagent (BioMol Cat. No. AK-111).

Phosphatase Assay

1. Prepare a fresh batch of Phosphatase Dilution Buffer and keep on ice.
2. Prepare phosphate standard curve following the instructions for BIOMOL Green Reagent. Briefly, prepare 1:1 serial dilutions of phosphate standard solutions with Phosphatase Dilution Buffer in a volume of 25 μl. Also, use 25 μl Phosphatase Dilution Buffer as a blank. The range of phosphate amount should be 0–4 nmole.
3. Thaw the active CDC25C and Phosphate Dilution Buffer on ice. Prepare serial dilutions of CDC25C using Phosphatase Dilution Buffer.
4. In a pre-cooled microcentrifuge tube, add the following reaction components in total volume of 25 μl:
   - 10 μl of Phosphatase Solution
   - 10 μl of Substrate Assay Solution
   - 5 μl of Phosphatase Dilution Buffer
5. Set up a blank control as outlined in step 4, substituting 10 μl of Phosphatase Dilution Buffer for the Phosphatase Solution.
6. Initiate each reaction by incubating the mixture in a water bath at 37 °C for 30 minutes.
7. Add 100 μl of BIOMOL Green Reagent to each reaction including control tubes.
8. Add 100 μl of BIOMOL Green Reagent to each phosphatase standard solution including blank.
9. Incubate all samples, controls, and standards at room temperature for 30 minutes to allow development of the green color.
10. Measure the absorbance of the reaction solution in a spectrophotometer at 630 nm.
11. Plot the free phosphate standard curve. Determine absorbance (y) for each sample (where y = absorbance of sample-background absorbance) and calculate the corresponding nmole of phosphate released (x) during the assay using the equation

    $y = A*x + B$ or $x = [y-B]/A$
    (the A and B values are determined from the slope of the line from the standard curve).
12. Calculate the phosphatase specific activity (SA)

Calculations:

1. Specific Phosphatase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{x\ (1000)}{T \times E}$$

x - corresponding phosphate released
T - reaction time (min)
E - Enzyme amount (μg)

References

1. Gould, K.L. et al., Complementation of the mitotic activator, p80(cdc25), by a human protein-tyrosine phosphatase. Science, **250**, 1573-1576 (1990).
2. Bulavin, D.V. et al., Initiation of a G_2/M checkpoint after ultraviolet radiation requires p38 kinase. Nature, **411**, 102-107 (2001).

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