

Product Information

CDC25B, active, GST tagged, human recombinant, expressed in Sf9 cells

Catalog Number **SRP5006**
Storage Temperature -70°C

Synonym: cell division cycle 25 homolog B

Product Description

CDC25B (also known as cell division cycle 25 homolog B) is a member of the CDC25 family of phosphatases that activate the cyclin dependent kinase CDC2 by removing two phosphate groups. CDC25B is required for cell entry into mitosis. CDC25B shuttles between the nucleus and the cytoplasm due to nuclear localization and nuclear export signals. Cdc25B is the first genetic model for studying the mechanisms regulating prophase arrest in vertebrates.¹ The regulation of CDC25B phosphorylation by p38 is a critical event for initiating the G₂/M checkpoint after ultraviolet radiation.²

Recombinant, full-length, human CDC25B was expressed in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_021873. 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: ~110 kDa

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

Specific Activity: 18–24 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)

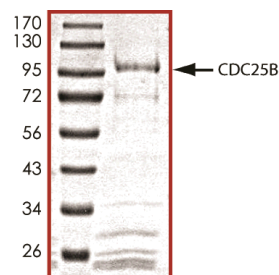
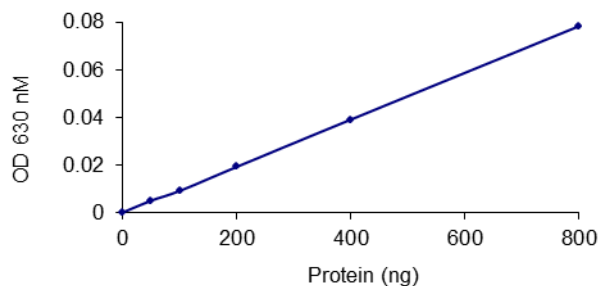


Figure 2.
Specific Activity of Typical Lot
18–24 nmol/min/mg



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 CDC25B (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 CDC25B 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 CDC25B and Phosphate Dilution Buffer on ice. Prepare serial dilutions of CDC25B 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

$$y = A \cdot x + B \text{ or } x = [y - B] / A$$

(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. Lincoln, A.J. et al., Cdc25b phosphatase is required for resumption of meiosis during oocyte maturation. *Nature Genet.*, **30**, 446-449 (2002).
2. Bulavin, D.V. et al., Initiation of a G₂/M checkpoint after ultraviolet radiation requires p38 kinase. *Nature*, **411**, 102-107 (2001).

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