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Product Information

CDC25A, active, GST tagged, human recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5005**
Storage Temperature –70 °C

Synonym: CDC25A2

Product Description
CDC25A (also known as cell division cycle 25 homolog A) is a member of the CDC25 family of phosphatases that is required for progression from G_1 to the S phase of the cell cycle. CDC25A can activate the cyclin-dependent kinase CDC2 (also known as CDK1) by removing two phosphate groups.[1] CDC25A is specifically degraded in response to DNA damage, which prevents cells with chromosomal abnormalities from progressing through cell division. CDC25A overexpression is detected in human cancers and this may contribute to the tumorigenesis process.[2] CDC25A is degraded by moderate heat shock and this degradation is protected by HSP90.

Recombinant, full-length, human CDC25A was expressed in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_001789. 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: ~94 kDa

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

Specific Activity: 26–36 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)

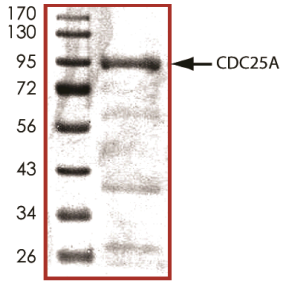
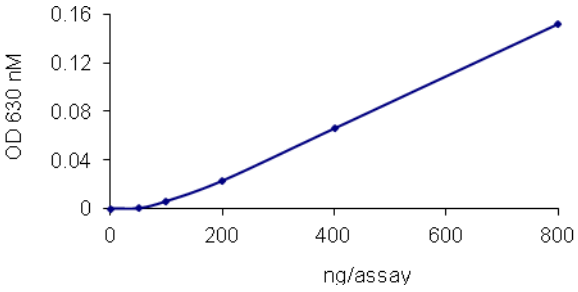


Figure 2.
Specific Activity of Typical Lot
26–36 nmol/min/mg



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 CDC25A (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 CDC25A 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 CDC25A and Phosphate Dilution Buffer on ice. Prepare serial dilutions of CDC25A 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. Mailand, N. et al., Rapid destruction of human Cdc25A in response to DNA damage. Science, **288**, 1425-1429 (2000).
2. Madlener, S. et al, Short 42 degrees C heat shock induces phosphorylation and degradation of Cdc25A which depends on p38MAPK, Chk2 and 14.3.3. Hum. Molec. Genet., **18**, 1990-2000 (2009).

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