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Catalogue #	Aliquot Size
C04-20G-05	5 µg
C04-20G-10	10 µg
C04-20G-20	20 µg

CDC25A, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog # C04-20G

Lot # I073-2

Product Description

Full-length recombinant human CDC25A was expressed in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_001789.

Gene Aliases

CDC25A2

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

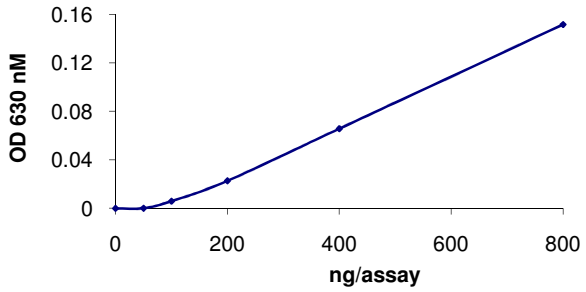
Scientific Background

CDC25A (also known as cell division cycle 25 homolog A) is a member of the CDC25 family of phosphatases that are required for progression from G1 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.

References

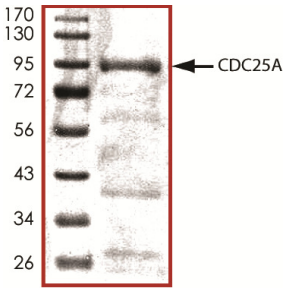
1. Mailand, N. et.al: Rapid destruction of human Cdc25A in response to DNA damage. Science 288: 1425-1429, 2000.
2. Madlener, S. et.al: Short 42 degrees C heat shock induces phosphorylation and degradation of Cdc25A which depends on p38MAPK, Chk2 and 14.3.3. Hum. Molec. Genet. 18: 1990-2000, 2009

Specific Activity



The specific activity of CDC25A was determined to be **31 nmol phosphate released /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>80%** by densitometry.
Approx. MW **94kDa**.

CDC25A, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog Number	C04-20G
Specific Activity	31 nmol/min/mg
Specific Lot Number	I073-2
Purity	>80%
Concentration	0.1µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Phosphatase (Catalog #: C04-20G)

Active CDC25A (0.1μg/μl) diluted with CDC25 Dilution Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active CDC25A for optimal results).

CDC25 Dilution Buffer

CDC25 Dilution Buffer contains 100mM TRIS-HCl, pH8.2, 40mM NaCl, 1mM dithiothreitol, 20% glycerol.

Substrate Assay Solution

OMFP (3-o-methylfluorescein phosphate) diluted in CDC25 Dilution Buffer to a final concentration of 500 μM.

Detection Solution

BIOMOL GREEN reagent phosphatase detection kit (BioMol Catalog #: AK-111).

Assay Protocol

Step 1. Prepare a fresh batch of CDC25 Dilution Buffer and keep on ice.

Step 2. Prepare phosphate standard curve following the instruction of BIOMOL GREEN reagent phosphatase detection kit. Briefly, prepare 1:1 serial dilutions of phosphate standard solutions with CDC25 Dilution Buffer in a volume of 25μl. Also, use 25μl CDC25 Dilution Buffer as a blank. The range of phosphate amount should be 0~4 nmol.

Step 3. Thaw the Active CDC25A on ice. Prepare serial dilutions of CDC25A using CDC25 Dilution Buffer.

Step 4. In a pre-cooled microfuge tube, add the following reaction components in total volume of 25μl:

Component 1. 10μl of diluted Active CDC25A (Catalog #C04-20G)
Component 2. 10μl of Substrate Assay Solution
Component 3. 5μl CDC25 Dilution Buffer

Step 5. Set up the blank control as outlined in step 4, excluding the addition of the Active Phosphatase. Replace the Active Phosphatase with an equal volume of CDC25 Dilution Buffer.

Step 6. Start the reaction by incubating the mixture in a water bath at 37°C for 30 minutes.

Step 7. Add 100μl BIOMOL GREEN Reagent to each reaction including control tubes.

Step 8. Add 100μl BIOMOL GREEN Reagent to each phosphate standard solution including the blank (step 2).

Step 9. Incubate at room temperature for 30 minutes to allow development of the green color

Step 10. Measure the absorbance of the reaction solution in a spectrophotometer at 630 nm.

Step 11. Plot the free phosphate standard curve. Determine absorbance (y) for each sample (where y = absorbance of sample – background absorbance) and calculate the corresponding nmol 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).

Step 12. Calculate the phosphatase specific activity (SA):

Phosphatase Specific Activity (SA) (nmol/min/mg)

SA = Corresponding phosphate released *1000/ [(Reaction time in min)*(Enzyme amount in μg)]

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