#7521 Store at –80°C

CDK2/CycA Kinase

☑ 5 µg

Cell Signaling TECHNOLOGY®

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new 08/05

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full-length human CDK2/CycA kinase, supplied as a GST fusion protein.

Background: Cyclins and cyclin-dependent kinases (CDK) are key regulators in mammalian cell cycle. Regulation of these complexes occurs through cyclin production and destruction, relocation, inhibitory and activating phoshorylation events, relocation, and also via the effects of other proteins. Each cyclin associates with one or two CDKs, and most CDKs associate with one or two cyclins (1,2,3). CDK1 forms a complex with Cyclin A/B and regulates phosphorylation of cytoskeleton proteins involved in mitosis. CDK2 and CDK3 form complexes with Cyclin E which regulate the G1-S phase transition while the CDK2/CycA complex regulates S phase progression (4,5). CDK4/CycD and CDK6/CycD are activated by mitogenic signaling during early G1 and progressively accumulate as cells transition through this phase of the cell cycle. CDK5 is activated in postmitotic neurons and regulates neuron migration during brain development (6). CDK7/CycH is believed to be a link between transcription and cell cycle. CDK8/CycC and CDK9/CycT are involved in transcription (1,2). The kinase activity of CDKs is tightly regulated by phosphorylation and protein-protein interactions. Activation of CDKs requires binding to a specific cyclin and phosphorylation of a conserved threonine residue in a region called the T loop. Examining the phosphorylation of peptides by CDK/Cyclin complexes suggests that both CDKs and cyclins play a role in recognizing substrates. A consensus sequence, (S/T)PX(R/K), is identified in the peptides that are phosphorylated by CDK/cyclins.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human CDK2 (Met1-Leu298) (GenBank Accession No. X62071) and full length human Cyclin A (Met1-Leu432) (GenBank Accession No. NM_001237), both with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-CDK2 fusion protein is 60 kDa. The theoretical molecular weight of the GST-Cyclin A fusion protein is 75 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain and Western blot [Fig.1]. CDK2/CycA kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure CDK2/CycA activity using HTScan™ CDK2/CycA Kinase Assay Kit #7522 [Fig.3].

Background References:

(1) Schang, L.M. (2002) *J. Antimicrob. Chemother.* 50, 779–792.
(2) Murray, A.W. (2004) *Cell* 116, 221–234.
(3) Chow, J.P. et al. (2003) *J. Biol. Chem.* 278, 40815–40828.
(4) Hofmann, F. and Livingston, D.M. (1996) *Genes Dev.* 10, 851–861.
(5) Golsteyn, R.M. (2005) *Cancer Lett.* 217, 129–138.
(6) Xie, Y. and Tsai, L.H. (2004) *Cell Cycle* 3, 108–110.
(7) Holmes, J.K. and Solomon, M.J. (1996) *J. Biol. Chem.* 271, 25240–25246.

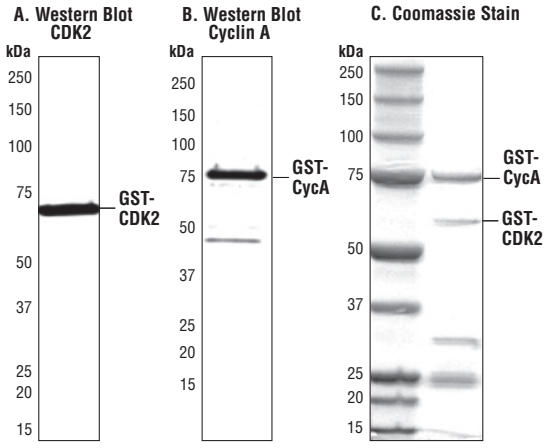

Figure 1. The purity of the GST-CDK2/CycA fusion protein was analyzed using SDS/PAGE followed by anti-CDK2 (A) and anti-CycA (B) Western blots or Coomassie stain (C).

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at -80 C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
HTScan™ CDK2/CycA Kinase Assay Kit #7522

Rb (Ser807/811) Biotinylated Peptide #1144

Phospho-Rb (Ser807/811) Antibody #9308

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to:
drugdiscovery@cellsignal.com

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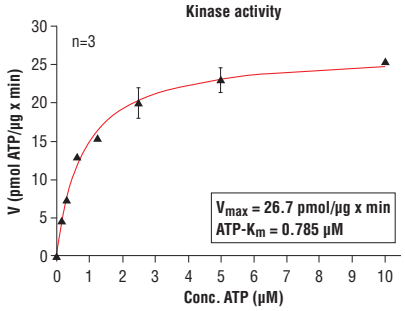

Figure 2. CDK2/CycA kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20.000, Substrate: Histone H1, 1 µg /50 µl, and 50 ng/50 µl Recombinant CDK2/CycA.

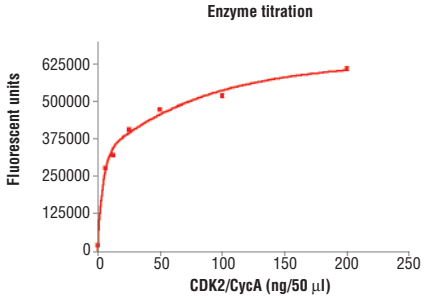

Figure 3. Dose dependence curve of CDK2/CycA kinase activity: DELFIA® data generated using Phospho-Rb (Ser807/811) Antibody #9308 to detect phosphorylation of substrate peptide (#1144) by CDK2/CycA kinase. In a 50 µl reaction, increasing amounts of CDK2/CycA and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

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Protocol for CDK2/CycA Kinase Assay

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

Additional Solutions and Reagents (Not included)

- Wash Buffer: 1X PBS, 0.05% Tween-20 (PBS/T)
- Bovine Serum Albumin (BSA)
- Stop Buffer: 50 mM EDTA pH 8
- Phospho-Rb (Ser807/811) Antibody #9308
- Kinase Buffer (10X) #9802
- ATP (10 mM) #9804
- Rb (Ser807/811) Biotinylated Peptide #1144
- DELFIA® Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
- DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
- DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

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Suggested Protocol For 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate]=3 µm).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 to make 2.5 ml 4X reaction buffer.
5. Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail ([enzyme]=4.0 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

25 mM Tris-HCl (pH7.5)

10 mM $MgCl_2$

5 mM β-glycerophosphate

0.1 mM Na_3VO_4

2 mM DTT

200 µM ATP

1.5 µM peptide

50 ng CDK2/CycA Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.

    Please note: This protocol was validated using a Rb (Ser807/811) Biotinylated Peptide and Phospho-Rb (Ser807/811) Antibody diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
13. Incubate at 37°C for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled secondary antibody 1:1000 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

***IMPORTANT: Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.**

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information. Email: drugdiscovery@cellsignal.com

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