Store at -80°C

#7522

# HTScan® CDK2/CycA Kinase Assay Kit

☑ 100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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| Products Included | Products # | Kit Quantity |
|---|---|---|
| Phospho-Rb (Ser807/811) Antibody | 9308 | 30 µl |
| Kinase Buffer (10X) | 9802 | 15 ml |
| ATP (10 mM) | 9804 | 1 ml |
| Rb (Ser807/811) Biotinylated Peptide | 1144 | 1.25 ml |
| CDK2/CycA Kinase (recombinant, human) | 7521 | 5 µg |

**Description:** The kit provides a means of performing kinase activity assays with recombinant human CDK2/CycA kinase. It includes active CDK2/CycA kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-serine/threonine antibody for detection of the phosphorylated form of the substrate peptide.

**Peptide Core Sequence:** IS*PLKS*P

**Molecular Weights:** Peptide substrate, Biotin-Rb (Ser807/811): GSK-CDK2: 60kDa, GST-CycA: 75 kDa.

**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 (1-3). 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.

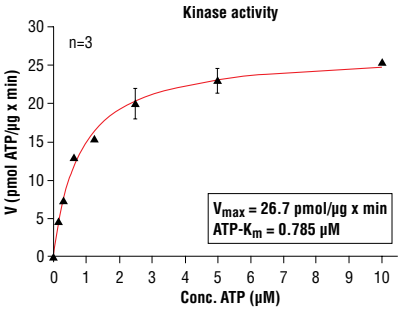


*Figure 1. CDK2/CycA kinase activity was measured in a radioisotopic filter binding assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: Histone H1, 1 µg /50 µl, recombinant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

**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 substrate peptide was selected using our Serine/Threonine Kinase Substrate Screening Kit #7400. Phospho-Rb (Ser807/811) Antibody #9308 was used for detection. The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

Purified CDK2/CycA kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain and Western blot. The specific activity of the CDK2/CycA kinase was determined using a radiometric assay [Fig.1]. Time course [Fig.2], kinase dose dependency [Fig.3] and substrate dose-dependency [Fig.4] assays were performed to verify CDK2/CycA activity using the CDK2/CycA substrate peptide provided in this kit. CDK2/CycA sensitivity to the inhibitor staurosporine was measured using the CDK2/CycA substrate peptide provided in this kit [Fig.5].

**Storage:** Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Peptides are supplied at 6 µM in 0.001% DMSO. Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

**Companion Products:**
Phospho-Rb (Ser807/811) Antibody #9308

Rb (Ser807/811) Biotinylated Peptide #1144

CDK2/CycA Kinase #7521

Serine/Threonine Kinase Substrate Screening Kit #7400

Staurosporine #9953

**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.

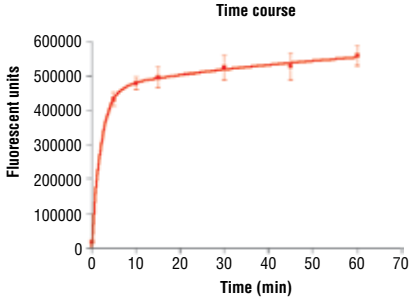


*Figure 2. Time course of CDK2/CycA kinase activity: DELFIA® data generated using Phospho-Rb (Ser807/811) Antibody #9308 to detect phosphorylation of substrate peptide (#1144) by CDK2/CycA kinase. In a 50 µl reaction, 50 ng CDK2/CycA and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)*

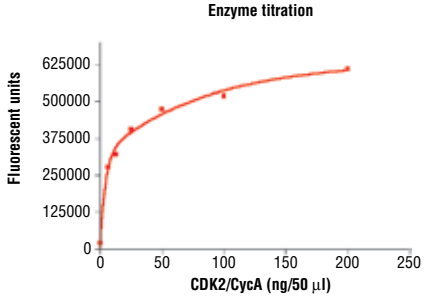


*Figure 3. Dose dependence curve of CDK2/CycA kinase activity: DELFIA® data generated using Phospho-Rb (Ser807/811) Antibody #9308 to detect phosphorylation of substrate peptide (#1144) by CDK2/CycA kinase. In a 50 µl reaction, increasing amounts of CDK2/CycA and 1.5 µM substrate peptide were used per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

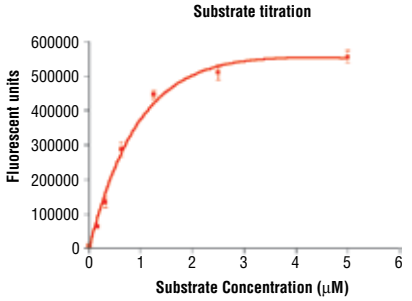


*Figure 4. Peptide concentration dependence of CDK2/CycA kinase activity: DELFIA® data generated using Phospho-Rb (Ser807/811) Antibody #9308 to detect phosphorylation of substrate peptide (#1144) by CDK2/CycA kinase. In a 50 µl reaction, 50 ng of CDK2/CycA and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)*

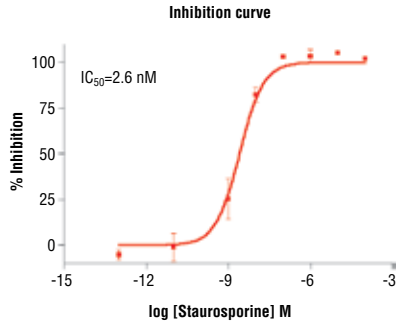


*Figure 5. Staurosporine inhibition of CDK2/CycA kinase activity: DELFIA® data generated using Phospho-(Ser807/811) Antibody #9308 to detect phosphorylation of substrate peptide (#1144) by CDK2/CycA kinase. In a 50 µl reaction, 50 ng CDK2/CycA, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)*

# Protocol for HTScan® CDK2/CycA Kinase Assay Kit

## 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.

## A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8

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## B 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 [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. Transfer 0.6 ml of 4X Reaction buffer to each enzyme tube to make 4X reaction cocktail ([enzyme]) = 8 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 (pH 7.5)
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, Phospho-Rb (Ser807/811) Antibody, 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. For DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

## DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a Time Resolved Fluorescent plate reader using the following settings;
   - **a.** Excitation Filter: 340 nm
   - **b.** Emission Filter: 615 nm
   - **c.** Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

**Companion Products for DELFIA®**

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

## Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 405 nm with a microtiter plate reader.

**Companion Products For Colorimetric ELISA Assay**

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

***NOTE:** 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