#7528 Store at –80°C

HTScan™ CDK3/CycE Kinase Assay Kit

☑ 100 Assays
(96 Well Format)

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.

Products Included	Product #	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
CDK3/CycE Kinase	7527	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human CDK3/CycE kinase. It includes active CDK3/CycE 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): 2,161 Daltons. GST-CDK3 kinase: 68 kDa, GST-CycE: 72 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 (1,2,3). 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.

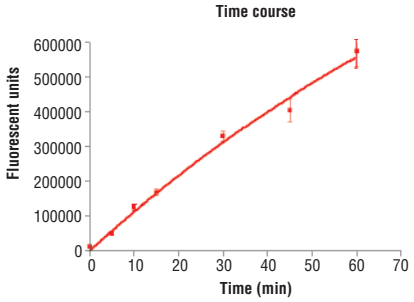

Figure 1. Time course of CDK3/CycE kinase activity: DELFIA® data generated using Phospho-Rb (Ser807/811) Antibody #9308 to detect phosphorylation of substrate peptide (#1144) by CDK3/CycE kinase. In a 50 µl reaction, 50 ng CDK3/CycE and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human CDK3 (Met1-His305) (GenBank Accession No. NM_001258) and full length human cyclin E (Met1-Ala395) (GenBank Accession No. M73812), 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 CDK3/CycE kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain and Western blot. Time course [Fig.1], kinase dose-dependency [Fig.2] and substrate dose-dependency [Fig.3] assays were performed to verify CDK3/CycE activity using the CDK3/CycE substrate peptide provided in this kit. CDK3/CycE kinase V_{max} and K_m values were determined using a radiometric filter binding assay [Fig.4]. CDK3/CycE sensitivity to the inhibitor staurosporine was measured using the CDK3/CycE 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:
Serine/Threonine Kinase Substrate Screening Kit #7400

Kinase Buffer (10X) #9802

CDK3/CycE Kinase #7527

Phospho-Rb (Ser807/811) Antibody #9308

Rb (Ser807/811) Biotinylated Peptide #1144

Staurosporine #9953

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

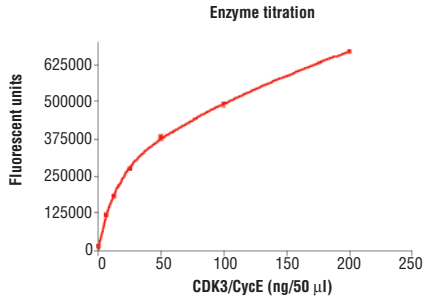

Figure 2. Dose dependence curve of CDK3/CycE kinase activity: DELFIA® data generated using Phospho-Rb (Ser807/811) Antibody #9308 to detect phosphorylation of substrate peptide (#1144) by CDK3/CycE kinase. In a 50 µl reaction, increasing amounts of CDK3/CycE and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

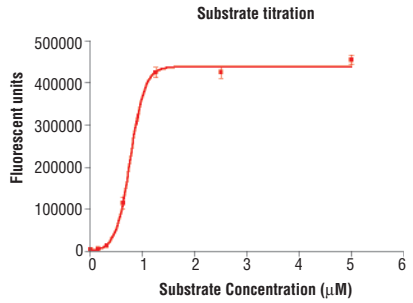

Figure 3. Peptide concentration dependence of CDK3/CycE kinase activity: DELFIA® data generated using Phospho-Rb (Ser807/811) Antibody #9308 to detect phosphorylation of substrate peptide (#1144) by CDK3/CycE kinase. In a 50 µl reaction, 50 ng of CDK3/CycE and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

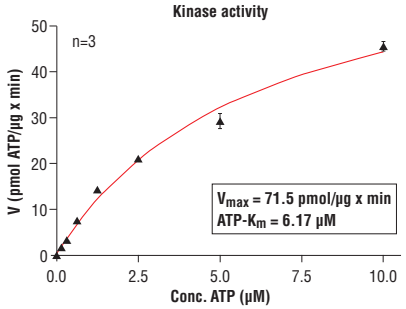

Figure 4. CDK3/CycE kinase activity was measured in a radioisotopic filter binding assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: Rb CTF, 5 µg /50 µl, Recombinant CDK3/CycE: 10 ng/50 µl.

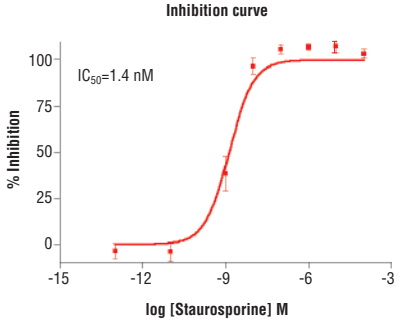

Figure 5. Staurosporine inhibition of CDK3/CycE kinase activity: DELFIA® data generated using Phospho-Rb (Ser807/811) Antibody #9308 to detect phosphorylation of substrate peptide (#1144) by CDK3/CycE kinase. In a 50 µl reaction, 50 ng CDK3/CycE, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

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.

Protocol for HTScan™ CDK3/CycE 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.

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
- 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 [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

200 µM ATP

2 mM DTT

1.5 µM peptide

50 ng CDK3/CycE 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 #9308, 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at 37°C for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled anti-rabbit 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