

SIGNAL TRANSDUCTION RESOURCE

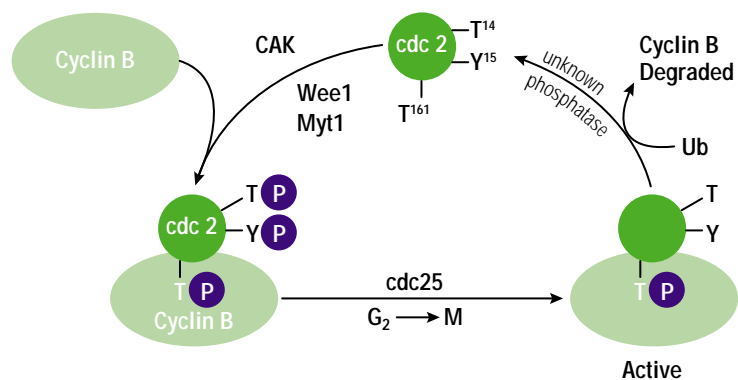


Figure 7.1. A simplified scheme for activation of cdc2 protein kinase. The catalytic subunit of cdc2 protein kinase (cdc2) is phosphorylated at T¹⁴, Y¹⁵ and T¹⁶¹ by CDK-activating kinase (CAK), Wee1 and Myt1. The phosphorylated cdc2 forms a complex with its regulatory subunit cyclin B. The protein kinase is inactive until cdc25 dephosphorylates T¹⁴ and Y¹⁵ during the transition from G₂ to M phase. The cdc2 kinase is inactivated by ubiquitin (Ub)-dependent degradation of cyclin B and dephosphorylation of T¹⁶¹ by an unknown phosphatase.

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cdc2 Kinase

Cyclin-dependent protein kinases (CDKs) are a family of serine/threonine kinases that mediate many stages of mitosis. Members of this family include cdc2 (also known as cdk1), cdk2-cdk7 and the RbKs. In mammalian cells, p34^{cdc2} regulates entry into mitosis (i.e., the transition from G2 to M phase) by phosphorylating a group of key proteins including RNA polymerase II and histone H1 (1,2). Phosphorylation by cdc2 and MEK are required for the disassembly of the golgi apparatus in preparation for partitioning into the two cells after mitosis (3). Regulation of CDK activity is a complex process. CDK catalytic subunits must associate with a subset of regulatory subunits called cyclins to become active. Other regulatory proteins activate or inhibit CDK by phosphorylation, dephosphorylation or binding to CDK (4-7).

p34^{cdc2} is a 297 amino acid protein with a predicted molecular weight of 34,073 daltons and an apparent molecular weight of 34kDa by SDS-PAGE (8). Activation of p34^{cdc2} requires multiple steps (Figure 7.1). p34^{cdc2} is phosphorylated by the protein kinases Wee1 (9) and Myt1 (10) on Thr14 and Tyr15 and by CDK-activating kinase on Thr161 prior to its association with cyclin B (11). Cyclin B is a 433 amino acid protein with a predicted molecular weight of 48,337 but an apparent molecular weight by SDS-PAGE of 62kDa (8). The complex is inactive at this point because phosphorylation of residues 14 and 15 block the ATP binding site of the kinase. During the G2/M transition, cdc25 activates the kinase by dephosphorylating Thr14 and Tyr15 (1,11). Removal of cyclin B through ubiquitin-dependent degradation (12) and dephosphorylation of Thr161 inactivates the kinase again.

References

1. Nurse, P. (1990) *Nature* **344**, 503.
2. Hartwell, L.H. and Kastan, M.B. (1994) *Science* **266**, 1821.
3. Kano, F. *et al.* (2000) *J. Cell Biol.* **149**, 357.
4. Lewin, B. (1990) *Cell* **61**, 743.
5. King, R.W. *et al.* (1994) *Cell* **79**, 563.
6. Hunter, T. and Pines, J. (1994) *Cell* **79**, 573.
7. Nigg, E.A. (1995) *BioEssays* **17**, 471.
8. Norbury, C. (1995) Cdc2 protein kinase (vertebrates). In: *The Protein Kinase Facts Book: Protein-Serine Kinases* (Hardie, G. and Hanks, S., eds.), Academic Press. 184.
9. McGowan, C.H. and Russell, P. (1995) *EMBO J.* **14**, 2166.
10. Booher, R.N., Holman, P.S. and Fattaey, A. (1997) *J. Biol. Chem.* **272**, 22300.
11. Morgan, D.O. (1995) *Nature* **374**, 131.
12. King, R.W. *et al.* (1996) *Science* **274**, 1652.

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Promega Product Citations

Hirota, T. *et al.* (2000) Zyxin, a regulator of actin filament assembly, targets the mitotic apparatus by interacting with h-warts/LATS1 tumor suppressor. *J. Cell Biol.* **149**, 1073.

Knowles, L.M. and Milner, J.A. (2000) Diallyl disulfide inhibits p34^{cdc2} kinase activity through changes in complex formation and phosphorylation. *Carcinogenesis* **21**, 1129.

Thomas, H.C. *et al.* (1998) Prolonged cell-cycle arrest associated with altered cdc2 kinase in monocrotaline pyrrole-treated pulmonary artery endothelial cells. *Am. J. Respir. Cell Mol. Biol.* **19**, 129.

Promega Resources

Protocol

SignaTECT® cdc2 Protein Kinase Assay System.....**TB227**

Publications

Goueli, S. *et al.* (1996) SAM²® Biotin Capture Membrane and SignaTECT® Protein Kinase Assay Systems. *Promega Notes* **58**, 22.

Enzyme Assay System

SignaTECT® Protein Kinase Assay System

Product	Size	Catalog #
SignaTECT® cdc2 Protein Kinase Assay System	96 reactions	V6430

Description: Promega's SignaTECT® cdc2 Protein Kinase Assay System^(a) uses a biotinylated peptide substrate derived from histone H1 to measure cdc2 kinase activity directly in cell lysates. This substrate (PKTPKKAKKL) is selective for cdc2 and possibly cdk2 and cdk3, providing the specificity necessary to perform cdc2 kinase assays on a variety of biological samples including crude cell lysates (1). The radiolabeled, phosphorylated, biotinylated substrate is recovered from the reaction mix with the SAM²® Biotin Capture Membrane^(a), which is a novel streptavidin matrix (2). The SAM²® Membrane is prenumbered and partially cut so that individual squares can be easily identified, separated and placed in scintillation vials. Alternatively, the intact SAM²® Membrane can be analyzed using a phosphorimaging system or by conventional autoradiography. Olomoucine, a specific inhibitor of a subset of cdk enzymes including cdc2, cdk2 and cdk5 (3), also is provided in the system and can be used to confirm the specificity of the reaction. With this assay, the analysis of cdc2 kinase is simple, rapid and amenable to high throughput. The assay detects olomoucine-sensitive cdc2 kinase activities at levels approaching pmol/min/μg of mitotic cell lysate protein.

^(a)U.S. Pat. No. 6,066,462 has been issued to Promega Corporation for quantitation of protein kinase activity.

References

1. Beaudette, K.N. *et al.* (1993) Substrate specificity characterization of a cdc2-like protein kinase purified from bovine brain. *J. Biol. Chem.* **268**, 20825.
2. Goueli, B.S. *et al.* (1995) A novel and simple method to assay the activity of individual protein kinases in a crude tissue extract. *Anal. Biochem.* **225**, 10.
3. Vesely, J. *et al.* (1994) Inhibition of cyclin-dependent kinases by purine analogues. *Eur. J. Biochem.* **224**, 771.

Features

- **High Signal-to-Noise Ratios:** The high specificity and strong affinity of the SAM²® Membrane for biotinylated substrates results in lower background and higher signal-to-noise ratios than traditional capture methods (i.e., P-81 phosphocellulose).
- **Linear Binding:** Membrane can linearly bind biotinylated substrates up to the nmol/cm² range—allows for kinetic studies.
- **Convenient:** SignaTECT® Systems require less “hands-on” manipulation than other assay methods.
- **Versatile:** The SAM²® Membrane can be used in a variety of buffer and reaction conditions.

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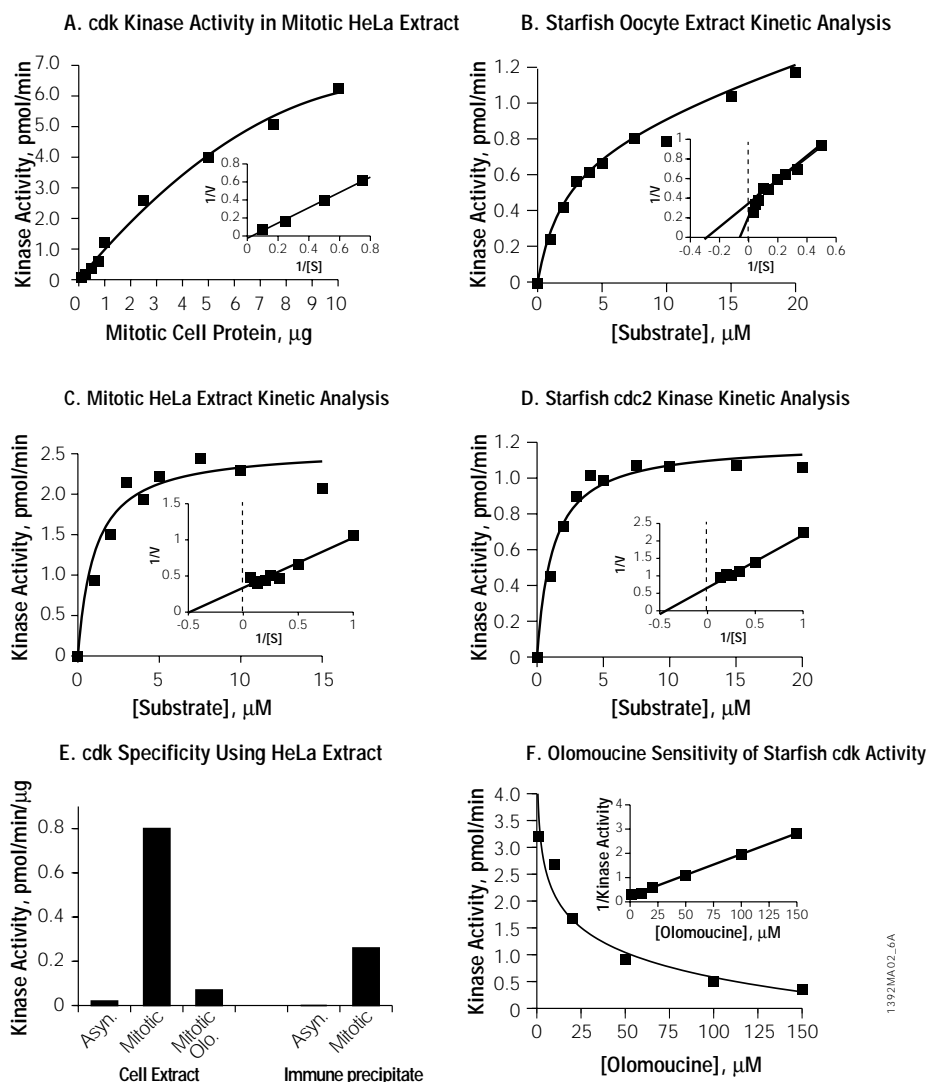


Figure 7.2. Analysis of cdc2 activities using the SignaTECT® cdc2 Kinase Assay System. Except where indicated, the cdc2 kinase assays were performed as described in Technical Bulletin #TB227. **Sensitivity Determination (A).** The indicated amounts of cell lysate prepared from HeLa cells, blocked in mitosis, were assayed for 15 minutes using 50 μ M ATP and 1 μ Ci of [γ - 32 P]ATP/reaction. Results were obtained with less than 1 μ g of extract protein. **Kinetic Analysis of Extracts.** Activated starfish oocyte extract (B) and mitotic HeLa cell extract (C) were incubated for 10 minutes with increasing concentrations of the peptide substrate. Double reciprocal plots are shown. An apparent K_m value of 2.0 μ M was observed in HeLa extracts while a biphasic curve with apparent K_m values of 3.3 μ M and 24 μ M was observed in starfish oocyte extracts. **Kinetic Analysis of Purified cdc2 Kinase (D).** cdc2 kinase purified from starfish was incubated with increasing concentrations of the peptide substrate. A double reciprocal plot with a K_m value of 2.3 μ M is shown. **Specificity (E).** Extracts prepared from actively growing HeLa cells and HeLa cells blocked in mitosis were analyzed for cdc2 kinase activity. Activity inhibited by 100 μ M Olomoucine, a selective inhibitor of cdc2 Kinase, was only detected in mitotic cell extracts. To confirm the specificity, 10 μ g of extract protein was immunoprecipitated with an anti-pp34^{cdc2} kinase C-terminus polyclonal antibody and protein A agarose. Approximately one-third of the cdc2 kinase activity was recovered in the immunoprecipitates. **Inhibition (F).** Activated starfish oocyte extract was incubated with 50 μ M ATP and the indicated concentrations of Olomoucine (Cat.# V2372). The single reciprocal plot indicates simple inhibition kinetics in the range reported for cdc2 kinase.

Note: Results presented here were obtained using a different assay buffer than that supplied with this system. The composition of the buffer used to obtain these data is 25mM MOPS, 10mM MgCl₂, 2mM EDTA, 1mM DTT, 40mM β -glycerophosphate, 20mM *p*-nitrophenylphosphate, 0.1mM sodium vanadate. The buffer supplied with this system provides improved reaction conditions for assaying cdc2 kinase activity, as measured in both extracts and purified enzyme, and thus optimized measurement of cdc2 kinase activity.

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Sapir, T. *et al.* (1999) LIS1 is a microtubule-associated phosphoprotein. *Eur. J. Biochem.* **265**, 181.

Antonsson, B. *et al.* (1998) Identification of in vitro phosphorylation sites in the growth cone protein SCG10. Effect of phosphorylation site mutants on microtubule destabilizing activity. *J. Biol. Chem.* **273**, 8439.

Kotani, S. *et al.* (1998) PKA and MPF-activated Polo-like kinase regulate anaphase-promoting complex activity and mitosis progression. *Mol. Cell* **1**, 371.

cdc2 Peptide Substrate

Harvey, K.J., Lukovic, D. and Ucker, D.S. (2000) Caspase-dependent Cdk activity is a requisite effector of apoptotic death events. *J. Cell Biol.* **148**, 59.

Olomoucine

Schang, L.M., Phillips, J. and Schaffer, P.A. (1998) Requirement for cellular cyclin-dependent kinases in herpes simplex virus replication and transcription. *J. Virol.* **72**, 5626.

Renzi, L. *et al.* (1997) MPM-2 antibody-reactive phosphorylations can be created in detergent-extracted cells by kinetochore-bound and soluble kinases. *J. Cell Sci.* **110**, 2013.

Enzymes, Substrates and Inhibitors

cdc2 Kinase, Human, Recombinant

Product	Size	Catalog #
cdc2 Kinase, Human, Recombinant	100 units	V2891

Description: cdc2 Kinase (cell cycle-dependent protein kinase), Human, Recombinant, is a serine/threonine protein kinase that acts as a universal trigger for mitosis and meiosis in eukaryotes. cdc2 Kinase is composed of 2 subunits: the catalytic subunit, cdc2 (34kDa), and the regulatory subunit, cyclin B (55kDa). Both subunits are required for activity. Human recombinant cdc2 is purified from *Spodoptera frugiperda* (Sf9) cells infected with a recombinant baculovirus carrying the genes for human p34^{cdc2} and cyclin B (1,2).

References

1. Parker, L.L. *et al.* (1992) p107wee1 is a dual-specificity kinase that phosphorylates p34^{cdc2} on tyrosine 15. *Proc. Natl. Acad. Sci. USA* **89**, 2917.
2. Atherton-Fessler, S. *et al.* (1993) Mechanisms of p34^{cdc2} regulation. *Mol. Cell. Biol.* **13**, 1675.

cdc2 Protein Kinase Peptide Substrate

Product	Size	Catalog #
cdc2 Protein Kinase Peptide Substrate	1mg	V2211

Description: cdc2 Protein Kinase Peptide Substrate is a peptide substrate for cdc2 protein kinase. This peptide is ready for use in kinase reactions. The sequence is derived from the pp34^{cdc2} in vitro phosphorylation sites of histone H1 (1). The sequence of cdc2 Protein Kinase Peptide Substrate is PKTPKKAKKL. Its molecular weight is 1,137 daltons as verified by Fast Atomic Bombardment mass spectrophotometry.

Reference

1. Parker, L.L. *et al.* (1992) p107wee1 is a dual-specificity kinase that phosphorylates p34^{cdc2} on tyrosine 15. *Proc. Natl. Acad. Sci. USA* **89**, 2917.

Olomoucine (cdc2 Protein Kinase Inhibitor)

Product	Size	Catalog #
Olomoucine (cdc2 Protein Kinase Inhibitor)	0.5mg	V2372
	10mg	V2373

Description: Olomoucine is a chemically synthesized inhibitor that is specific for pp34^{cdc2} and related protein kinases. Its molecular weight is 298 and its molecular formula is C₁₅H₁₈N₆O.

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