
Certificate of Analysis

cdk1/cdc2 Kinase Assay Kit

Catalog # 17-137

Lot # 31847

Kit Components

Assay Dilution Buffer I (ADBI), Catalog # 20-108. Two vials, each containing 1ml of ADBI: 20mM MOPS, pH 7.2, 25mM β -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.

cdc2 Substrate Cocktail, Catalog # 20-125. Two vials, each containing 1ml of substrate cocktail: 2mg/ml Histone H1 (Catalog # 14-155) in ADBI.

Inhibitor Cocktail, Catalog # 20-116. Two vials, each containing 1ml of inhibitor cocktail: 20 μ M PKC inhibitor peptide [Catalog #12-121], 2 μ M PKA inhibitor peptide (PKI) [Catalog #12-151], and 2 μ M Compound R24571 in ADBI.

Magnesium/ATP Cocktail, Catalog # 20-113. Two vials, each containing 1ml of Mg²⁺/ATP cocktail: 75mM magnesium chloride and 500 μ M ATP in ADBI. Before starting the assay, 90 μ l of the Magnesium/ATP cocktail should be added to 100 μ Ci (10 μ l) of [γ -³²P]ATP (~3000 Ci/mmol).

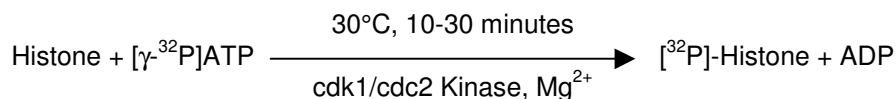
P81 Phosphocellulose Squares, Catalog # 20-134. One pouch containing 200 prelabeled squares.

Kit Description

Quantity: 200 kinase assays per kit.

Storage and Stability: Stable for 1 year at -20°C from date of shipment.

Use: The assay kit is designed to measure the phosphotransferase activity of cdk1/cdc2 Kinase in immunoprecipitates and column fractions. Crude cell lysates may also be used but detergents/biochemicals contained in the cell lysis buffer may inhibit cdk1/cdc2 kinase activity. Furthermore, although 3 inhibitors are included with the kit, editors may suggest other unknown kinases found in crude lysates are responsible for histone phosphorylation. The assay kit is based on phosphorylation of a specific substrate (histone) using the transfer of the γ -phosphate of adenosine-5'-[³²P] triphosphate ([γ -³²P] ATP) by cdk1/cdc2 Kinase. The phosphorylated substrate is then separated from the residual [γ -³²P] ATP using P81 phosphocellulose paper and quantitated by using a scintillation counter. The assay is linear for incubation times of up to 30 minutes and incorporation of up to 20% of total ATP. Further incubation or incorporation may not be linear and may therefore not be a true indication of cdk1/cdc2 Kinase activity in the sample extract. The enzyme assay is rapid and convenient. Each kit contains sufficient reagents for 200 individual cdk1/cdc2 Kinase assays.



FOR RESEARCH USE ONLY
NOT RECOMMENDED OR INTENDED FOR DIAGNOSIS OF DISEASE IN HUMANS
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Other components required but not included as part of kit are:

- **Enzyme Preparation containing cdk1/cdc2 Kinase** (10-200µg protein/immunoprecipitate diluted into ADBI. 200ng of cdk2/cyclin A, active (Catalog # 14-448) may also be used.
- vortex mixer
- plexiglass shielding
- 30°C incubating water bath
- timer
- variable volume (5-200µl) pipet + tips
- 0.75% phosphoric acid
- acetone
- scintillation vials
- scintillation fluid
- scintillation counter
- [γ -³²P]ATP - ~3000 Ci/mmol, obtained from PerkinElmer, Catalog # BLU002A.
- 50ml disposable conical centrifuge tubes

Safety Warnings and Precautions: The cdk1/cdc2 Kinase assay kit is designed for research use only and not recommended for diagnostic and internal use in humans or animals. Since the kit involves the use of radioactive [γ -³²P]ATP, please follow institutional procedures for handling, use, storage and disposal of such materials. All chemicals should be considered potentially hazardous and handled using good laboratory practice.

cdk1/cdc2 Assay Kit Procedures

The kit components should be thawed, mixed by vortexing and stored on ice before proceeding with the assay. In particular, ADBI and Magnesium/ATP cocktail must be rapidly thawed and mixed completely. Note: do not use extended thawing time. The assay components can be refrozen at -20°C for extended periods. Perform all preincubation reactions at 1°C over an ice bath. The kinase assay may be performed at 30°C or room temperature but linear results are more easily achieved at 30°C.

Suitable blanks should always be performed to correct for non-specific binding of [γ -³²P]ATP and its breakdown products to the phosphocellulose paper. Controls for endogenous phosphorylation of proteins in the sample extract can be performed by substituting assay dilution buffer for substrate cocktail.

Assay Procedure:

1. Add 10µl of the substrate cocktail to a microcentrifuge tube.
2. Add 10µl of the inhibitor cocktail or ADBI to a microcentrifuge tube.
3. Add 10µl of ADBI to the microcentrifuge tube.
4. Add 10µl of cdk1/cdc2 Kinase (200ng cdk2/cyclin A, active or 10-200µg protein/immunoprecipitate).
5. Add 10µl of the diluted [γ -³²P]ATP mixture.
6. Incubate for 10 minutes at 30°C with constant agitation.
7. Transfer a 25µl aliquot onto the center of a numbered P81 paper.
8. Wash the assay squares three times with 0.75% phosphoric acid.
9. Wash the assay squares once with acetone for 5 minutes.
10. Transfer the assay squares to a scintillation vial and add scintillation cocktail.
11. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain no enzyme (background control).

Technical Note: Allow the radiolabeled substrate to bind to the filter paper for 30 seconds before immersing the paper into a 50ml conical tube containing 40ml 0.75% phosphoric acid. Gently shake the assay squares for 5 minutes on a rotator. Discard the wash in a liquid radioisotope waste container, (dispose of per institutional regulations) and repeat the wash step twice. Wash the squares in 20ml of acetone for 5 minutes. Drain and add scintillation cocktail.

cdk1/cdc2 Kinase Assay Data: cdk1/cdc2 Kinase activity was measured using 200ng of cdk2/cyclin A, active (Catalog # 14-448). This kit uses Histone H1 as a kinase substrate and contains a separate inhibitor cocktail that blocks the activity of other serine/threonine kinases such as PKA, PKC and calmodulin dependent kinases. Test results are shown to the right:

cdk2/ cyclin A	Inhibitors Present	Substrate	Mean CPM	Comments
200ng (B)	PKA, PKC and CaMK	none	2,945	background phosphorylation
none	PKA, PKC and CaMK	Histone H1	3,651	substrate background
200ng (A)	PKA, PKC and CaMK	Histone H1	174,747	cdk1/cdc2 activity

Determination of cdk1/cdc2 Kinase Activity:

Determine the specific radioactivity of the Mg^{2+} /cold ATP-hot ATP mixture. Assume that the amount of hot ATP is negligible. In the above experiment, 5 μ l of the ATP solution gave 6.3 x 10⁶CPM, therefore 10 μ l would give 12.6 X 10⁶CPM = (500 μ M x 10⁻⁵ L x 10³nmoles/ μ mole = 5nmoles ATP).

$$12.6 \times 10^6 \text{ CPM} / 5 \text{ nmoles ATP}$$

$$2.52 \times 10^6 \text{ CPM/nmole ATP} = 2,520 \text{ CPM/pmol ATP}$$

= Specific Radioactivity (S.R.)

The [³²P] incorporated into the substrate is quantitatively measured by its binding to the phosphocellulose paper. In the presence of sample extract, the [³²P] counted on the paper is the sum of non-specific [γ -³²P]ATP binding, specific binding of phosphorylated substrate and binding of phosphorylated endogenous proteins in the sample extracts (A).

In the absence of substrate, the [³²P] counted on the papers is due to non-specific binding of [γ -³²P]ATP and its breakdown products and binding of phosphorylated endogenous proteins in the sample extracts (B).

Therefore, the [³²P] incorporated into the substrate is obtained from (A-B).

Since only 25 μ l of the incubation mixture was spotted onto the P81 paper out of a total volume of 50 μ l, the total [³²P] incorporated into the substrate is given by [A-B] X 2.

$$\frac{[A-B] \times 2}{\text{S.R.} \times 10 \text{ min}} = \text{pmol phosphate incorporated into histone/min/200ng of enzyme}$$

$$= \frac{[174,747 - 3,651] \times 2}{2,520 \times 10 \text{ minutes}}$$

$$= 13.6 \text{ pmol incorporated into histone/min/200ng}$$

$$= 0.068 \text{ pmol incorporated into histone/min/ng}$$

$$= 68 \text{ pmol incorporated into histone/min/}\mu\text{g}$$