Non-Radioisotopic Kit for Measuring Cdc2-Cyclin B Activity

CycLex Cdc2-Cyclin B Kinase Assay Kit

Cat# CY-1164

Intended Use

The CycLex Research Product **CycLex Cdc2-Cyclin B Kinase Assay Kit** is primarily designed to measure the activities of purified Cdc2-Cyclin B for the rapid and sensitive evaluation of inhibitors or activators. The phospho-specific monoclonal antibody used in this assay kit has been demonstrated to recognize the phospho-threonine 376 residue in human Cdc7, which is phosphorylated by Cdc2-Cyclin B kinase but not by Cdk2-Cyclin A, Cdk2-Cyclin E, Cdk4-Cyclin D and Cdk6-Cyclin D *in vitro*. Additionally, column fractions of any cultured primary cell, cell line, or tissue homogenate can be assayed for Cdc2-Cyclin B activity with the CycLex Research Product **CycLex Cdc2-Cyclin B Kinase Assay Kit** if the appropriate dose of Cdc2 kinase specific inhibitor, e.g. Purvalanol A, Roscovitine or Alsterpaullone, is used.

Applications of this kit include:
1) Monitoring the purification of Cdc2-Cyclin B kinase.
2) Screening inhibitors or activators of Cdc2-Cyclin B kinase.
3) Detecting the effects of pharmacological agents on Cdc2-Cyclin B kinase.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at 4°C.
- Don't expose reagents to excessive light.

Introduction

A few years after the identification of cdc2-cyclin B kinase as the universal factor that controls onset of M-phase in eukaryotic cells, MPF (M-phase promoting factor), it became evident that all transitions of the cell cycle are controlled through phosphorylation of specific targets due to changes in the activity of a variety of cyclin-dependent kinases (cdks). These transitions include conversion of quiescent cells to a state of active proliferation, commitment to DNA replication, initiation of DNA replication, and entry into and exit from mitosis. Changes in the activity of cdks along the cell cycle depend not only on their association with a variety of cyclins (including G1/S and G2/M cyclins) and on posttranslational modifications by phosphorylation-dephosphorylation reactions, but also on specific protein inhibitors and on protein degradation.

Although cdc2-cyclin B is thought to be the major kinase that initiates the onset of mitosis, a more complete understanding of how cells move from G2 to a mitotic state will require further identification of kinases operating upstream, downstream and in parallel with Cdc2, their substrates and their relationship with one another during the G2/M transition.

Measurement of Cdc2-Cyclin B kinase activity

The protocol generally regarded as most sensitive for the quantitative measurement of Cdc2-Cyclin B activity involves incubation of the Cdc2-Cyclin B sample with substrate, either a natural or synthetic polypeptide (such as Histone H1 substrate peptide), in the presence of Mg^{2+} and ^{32}P-labeled ATP. The reaction is terminated by "spotting" a sample onto a filter paper disc, followed by immersion in acid to precipitate the radiolabeled product. The filter papers are then washed extensively to remove unincorporated radiolabel and the radioactivity counted. While sensitive, this method is labor-intensive, generates hazardous radioactive waste and depends on a radioisotope of short half-life. It is particularly unsuitable when kinase assays are only performed on an infrequent basis. The **CycLex Cdc2-Cyclin B Kinase Assay Kit** uses an anti-phospho-Cdc7-Threonine376 monoclonal antibody and peroxidase coupled anti-mouse IgG antibody as a reporter molecule in a 96-well ELISA format. This assay provides a non-isotopic, sensitive and specific method to measure the activities of Cdc2-Cyclin B.

Principle of the Assay

The CycLex Research Products **CycLex Cdc2-Cyclin B Kinase Assay Kit** is a single-site, non-quantitative immunoassay for Cdc2-Cyclin B activity. Plates are pre-coated with a substrate corresponding to recombinant Cdc7, which contains threonine residue that can be phosphorylated by Cdc2-Cyclin B but not by Cdk2-Cyclin A, Cdk2-Cyclin E, Cdk4-Cyclin D and Cdk6-Cyclin D *in vitro*. The detector antibody specifically detects only the phosphorylated form of threonine residue on Cdc7.

The **CycLex Cdc2-Cyclin B Kinase Assay Kit** can be used to study the kinetics of a purified or partially purified Cdc2-Cyclin B as well as to screening Cdc2-Cyclin B inhibitor. To perform the test, the sample is diluted in Kinase Buffer, pipetted into the wells and allowed to phosphorylate the bound substrate following the addition of Mg^{2+} and ATP. The amount of phosphorylated substrate is measured by binding it with a TK-3H7, a anti-phospho-Cdc7-Threonine376 antibody, followed by binding with horseradish peroxidase conjugated anti-mouse IgG, which then catalyzes the conversion of the chromogenic substrate tetra-methylbenzidine (TMB) from a colorless solution to a blue solution (or yellow after the addition of stopping reagent). The color is quantitated by spectrophotometry and reflects the relative amount of Cdc2-Cyclin B activity in the sample. For kinetic analysis, the Cdc2-Cyclin B containing sample is added to the wells in a similar fashion and at varying times the reaction is stopped by the addition of the chelator, sodium ethylenediaminetetraacetate (EDTA) and the amount of phosphorylated substrate determined as before.

The CycLex Research Product **CycLex Cdc2-Cyclin B Kinase Assay Kit** is designed to accurately determine the presence and relative amount of Cdc2-Cyclin B Kinase activity in purification column fractions, and to determine non-isotopic kinetic analysis of Cdc2-Cyclin B Kinase activity. Careful attention to extraction methods and the assay protocol will provide the investigator with a reliable tool for the evaluation of Cdc2-Cyclin B.

Summary of Procedure

Add 100 µL of sample to the wells
↓ Incubate for 30 min at 30°C
Wash the wells
↓
Add 100 µL of Anti-phospho-Cdc7 threonine376 monoclonal antibody
↓ Incubate for 30 min at room temp.
Wash the wells
↓
Add 100 µL of HRP conjugated anti-mouse IgG
↓ Incubate for 30 min at room temp.
Wash the wells
↓
Add 100 µL of Substrate Reagent
↓
Add 100 µL of Stop Solution
↓
Measure absorbance at 450 nm

Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for the one 96-well microtiter plate kit.

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with recombinant Cdc7 as Cdc2-Cyclin B kinases substrate.

10X Wash Buffer: One bottle containing 100 mL of 10X buffer containing 2 %Tween®-20

Kinase Buffer: One bottle containing 20 mL of 1X buffer; used for Kinase Reaction Buffer and sample dilution.

20X ATP: One vial of lyophilized ATP Na_2 salt.

Anti-Phospho-Cdc7-T376 Monoclonal Antibody: One vial containing 12 mL of anti-phospho-Cdc7-Threonine376 monoclonal antibody (TK-3H7). Ready to use.

HRP conjugated Anti-mouse IgG: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-mouse IgG. Ready to use.

Substrate Reagent: One bottle containing 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.

Stop Solution: One bottle containing 20 mL of 1 N H_2SO_4. Ready to use.

Materials Required but not Provided

- **Cdc2-Cyclin B positive control:** Available from CycLex (Cdc2-Cyclin B positive control: Cat# CY-E1164, One vial containing 3 units/400 µL Cdc2-Cyclin B enzyme. Positive control should be added to the first well at 15 m units/well. For instance, diluted positive control 1:5, use 10 µL for 1 assay. (Unused Cdc2-Cyclin B enzyme should be stored in aliquots at -70°C.)
- **10X Purvalanol A (100 µM):** Purvalanol A is available from Sigma, Cat# P-4484. 10 mM stock solution (DMSO) diluted 1:100 in Kinase Buffer. Roscovitine and Alsterpaullone are also available from Sigma, Cat# is R-7772 and A-4847, respectively.
- **Pipettors:** 2-20 µL, 20-200 µL and 200-1000 µL precision pipettors with disposable tips.
- **Precision repeating pipettor**
- **Wash bottle or multichannel dispenser** for plate washing.
- **Microcentrifuge and tubes** for sample preparation.
- **Vortex mixer**
- **Plate reader** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **500 or 1000 mL graduated cylinder**
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**

Precautions and Recommendations

- Store the Cdc2-Cyclin B enzyme at -70° C and the ATP at -20°C when not in use. Store all other components at 4°C. Do not expose reagents to excessive light. Avoid freeze/thaw cycles.
- Allow all the components to come to room temperature before use.
- All microplate strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Use only the microtiter wells provided with the kit.
- Rinse all detergent residue from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents in this kit may contain preservatives or other chemicals. Care should be taken to avoid direct contact with these reagents.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with Substrate Solution which contains hydrogen peroxide.
- Avoid contact with Stop Solution which contains Sulfuric Acid.
- In case of contact with the Stop Solution and the Substrate Solution, wash skin thoroughly with water and seek medical attention, when necessary.
- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**
- **CAUTION: Sulfuric Acid is a strong acid. Wear disposable gloves and eye protection when handling Stop Solution.**

Detailed Protocol

The **CycLex Cdc2-Cyclin B Kinase Assay Kit** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since conditions may vary, running an aliquot of the appropriate Cdc2-Cyclin B positive control (Cat# CY-E1164), separately available from CycLex, should be included in each assay. Disposable pipette tips and reagent troughs should be used for all transfers to avoid cross-contamination of reagents or samples.

Preparation of Working Solution

1. Prepare a working solution of **Wash Buffer** by adding 100 mL of the **10X Wash Buffer** (provided) to 900 mL of ddH_2O. Mix well. Store at 4°C for two weeks or -20°C for long-term storage.

2. Prepare **20X ATP Solution** by adding **0.8 mL** of ddH_2O to the vial of **20X ATP** (provided, lyophilized). Mix gently until dissolved. the Final concentration of the **20X ATP Solution** should be **2.5 mM**. Store the solution in small aliquots (e.g. 100 µL) at -20°C.

3. Prepare **Kinase Reaction Buffer** by mixing following reagents.

	96 assays	10 assays	1 assay
Kinase Buffer (provided)	**9.5 mL**	**950 µL**	**95 µL**
20X ATP Solution	**0.5 mL**	**50 µL**	**5 µL**
Total	**10 mL**	**1000 µL**	**100 µL**

You will need 80-90 µL of Kinase Reaction Buffer per assay well. Mix well. Discard any unused Kinase Reaction Buffer after use.

Standard Assay

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.

2. Prepare all samples (diluted with Kinase Buffer as needed). All samples should be assayed in duplicate.

3. To assay partially purified recombinant Cdc2-Cyclin B, add **10 µL** of **each fraction** to the wells of the assay plate on ice. Duplicate wells 15 m units/10 µL of Cdc2-Cyclin B positive control (Cat # CY-E1164) should be included in each assay as a positive control for phosphorylation.

4. Begin the kinase reaction by addition of **90 µL Kinase Reaction buffer** per well, cover with plate sealer, and incubate **at 30°C for 30 minutes**.

5. Wash wells five times with Wash Buffer making sure each well is filled completely. Remove residual Wash Buffer by gentle tapping or aspiration.

6. Pipette **100 µL** of **Anti-phospho Cdc7 T376 Monoclonal Antibody** into each well, cover with a plate sealer and incubate **at room temperature (ca.25°C) for 30 minutes**.

7. Wash wells five times as same as in step 5.

8. Pipette **100 µL** of **HRP-conjugated Anti-mouse IgG** into each well, cover with plate sealer or lid, and incubate **at room temperature (ca.25°C) for 30 minutes**. Discard any unused conjugate after use.

9. Wash wells five times as same as in step 5.

10. Add **100 µL** of **Substrate Reagent** to each well and incubate **at room temperature (ca.25°C) for 5–15 minutes**.

11. Add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.

12. Measure absorbance in each well using a spectrophotometric plate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the plate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Note-1: Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Note-2: Reliable signals are obtained when either O.D. values do not exceed 0.25 units for the blank (no enzyme control), or 2.5 units for the Cdc2-Cyclin B positive control.

Note-3: If the microplate reader is not capable of reading absorbance greater than the absorbance of the Wee1 positive control, perform a second reading at 405 nm. A new O.D. values, measured at 405 nm, is used to determine Cdc2-Cyclin B activity of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Kinetic Assay

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.

2. Prepare all samples (diluted with Kinase Buffer as needed). All samples should be assayed in duplicate.

3. To assay partially purified recombinant Cdc2-Cyclin B, add **10 µL** of **each fraction** to the wells of the assay plate on ice. Duplicate wells 15 m units/10 µL of Cdc2-Cyclin B positive control (Cat # CY-E1164) should be included in each assay as a positive control for phosphorylation.

4. Begin kinase reaction by addition of **90 µL Kinase Reaction Buffer** in duplicate per well in timed intervals (suggested interval is 5 minutes but should be individually determined for each system). After the final addition, incubate **at 30°C for 20 minutes**.

5. Stop the reaction by flicking out the contents. (Alternatively, the reaction may be terminated by the addition of 150 µL 0.1 M Na EDTA, pH 8.0 to each well).

6. Wash wells five times with Wash Buffer making sure each well is filled completely. Remove residual Wash Buffer by gentle tapping or aspiration.

7. Pipette **100 µL** of **Anti-phospho Cdc7 T376 Monoclonal Antibody** into each well, cover with a plate sealer and incubate **at room temperature (ca.25°C) for 30 minutes**.

8. Wash wells five times as same as in step 6.

9. Pipette **100 µL** of **HRP-conjugated Anti-mouse IgG** into each well, cover with plate sealer or lid, and incubate **at room temperature (ca.25°C) for 30 minutes**. Discard any unused conjugate after use.

10. Wash wells five times as same as in step 6.

11. Add **100 µL** of **Substrate Reagent** to each well and incubate **at room temperature (ca.25°C) for 5–15 minutes**.

12. Add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.

13. Measure absorbance in each well using a spectrophotometric plate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the plate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Recommendations

Special considerations when screening activators or inhibitors

In order to estimate the inhibitory effect on individual Cdc2-Cyclin B activity in the test chemicals correctly, it is necessary to conduct the control experiment of "Solvent control" at least once for every experiment and "Inhibitor control" at least once for the first experiment, in addition to "Test sample", as indicated in the following table. When test chemicals cause an inhibitory effect on Cdc2-Cyclin B activity, the level of A450 is weakened as compared with "Solvent control". The high level of A450 is not observed in "Inhibitor control" (usually A450<0.3).

Assay reagents	Test sample	Solvent control	Inhibitor control
Kinase Reaction buffer	**80 µL**	**80 µL**	**80 µL**
10X Inhibitor or equivalent	**10 µL**	**-**	**-**
Solvent for Inhibitor	**-**	**10 µL**	**-**
10X Purvalanol A (100 µM)*	**-**	**-**	**10 µL**
Cdc2-Cyclin B positive control (1.5 m unit/µL) or your enzyme fraction**	**10 µL**	**10 µL**	**10 µL**

* 10X Purvalanol A: See Page 4, section "Materials Required but not Provided"
** Cdc2-Cyclin B positive control: Cat# CY-E1164: See Page 4, section "Materials Required but not Provided"

1. Following the above table, add the Reagents to each well of the microplate. Finally, initiate reaction by adding **10 µL** of **"Cdc2-Cyclin B positive control (1.5 m unit/µL)"** to each well and mixing thoroughly at room temperature. Cover with plate sealer or lid, and incubate **at 30°C for 30 minutes**.

2. Follow the **Standard Assay** steps 5-12, page 6-7.

Special considerations when measuring precise Cdc2-Cyclin B kinase activity

In order to measure the activity of Cdc2-Cyclin B correctly, it is necessary to conduct the control experiment of "Inhibitor control" at least once for every experiment and "ATP minus control" at least once for the first experiment, in addition to "No enzyme control" as indicated in the following table. Although the level of A450 increases in "Test sample" when Cdc2-Cyclin B enzyme activity is in the sample, the high level of A450 is not observed in "Inhibitor control", "ATP minus control" and "No enzyme control".

Assay reagents	**Test Sample**	**Inhibitor control**	**ATP minus control**	**Positive control**	**No enzyme control**
Kinase Reaction buffer	**90 µL**	**80 µL**	**-**	**90 µL**	**90 µL**
Kinase Buffer (provided)	**-**	**-**	**90 µL**	**-**	**-**
10X Purvalanol A (100 µM)*	**-**	**10 µL**	**-**	**-**	**-**
Your enzyme fraction	**10 µL**	**10 µL**	**10 µL**	**-**	**-**
Cdc2-Cyclin B positive control (1.5 m unit/µL)**	**-**	**-**	**-**	**10 µL**	**-**
Buffer	**-**	**-**	**-**	**-**	**10 µL**

* 10X Purvalanol A: See Page 4, section "Materials Required but not Provided"
** Cdc2-Cyclin B positive control: Cat# CY-E1164: See Page 4, section "Materials Required but not Provided"

1. Following the above table, add the Reagents to each well of the microplate. Finally, initiate the reaction by adding **10 µL** of **"Your enzyme fraction"** or **"Buffer"** to each well and mixing thoroughly at room temperature. Cover with plate sealer or lid, and incubate **at 30°C for 30 minutes**.

2. Follow the **Standard Assay** steps 5-12, page 6-7.

Evaluation of Results

1. Average the absorbance values for the Cdc2-Cyclin B sample duplicates (positive control) and all experimental sample duplicate values (when applicable). When Cdc2-Cyclin B positive control (15 m units/assay) is included as an internal control for the phosphorylation reaction, the absorbance value should be greater than 1.0 with a background less than 0.3.

2. For screening of purification/chromatography fractions, on graph paper, plot the mean absorbance values for each of the samples on the Y-axis versus the fraction number on the X-axis to determine the location of the eluted, purified Cdc2-Cyclin B.

3. For kinetic analysis, on graph paper, plot the mean absorbance values for each of the time points on the Y-axis versus the time of each reaction (minutes) on the X-axis.

Assay Characteristics

The CycLex Research Product **CycLex Cdc2-Cyclin B Kinase Assay Kit** has been shown to detect the activity of Cdc2-Cyclin B in column fractions of human or animal cell lysates. The assay shows good linearity of sample response. The assay may be used to follow the purification of Cdc2-Cyclin B.

Troubleshooting

1. The **CycLex Cdc2-Cyclin B positive control** (Cat# CY-E1164) should be run in duplicate, when a standard assay is being performed, using the protocol described in the "**Detailed Protocol**". Incubation times or temperatures significantly different from those specified may give erroneous results.

2. The reaction curve is nearly a straight line if the kinetics of the assay is of the first order. Variations in the protocol can lead to non-linearity of the curve, as can assay kinetics of other than first order. For a non-linear curve, point to point or quadratic curve fit methods should be used.

3. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate insufficient washing. If all instructions in the "**Detailed Protocol**" were followed accurately, such results indicate a need for washer maintenance.

4. Overall low signal may indicate that desiccation of the plate has occurred between the final wash and addition of Substrate Reagent. Do not allow the plate to dry out. Add Substrate Reagent immediately after wash.

Reagent Stability

All of the reagents included in the CycLex Research Product **CycLex Cdc2-Cyclin B Kinase Assay Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date. Upon receipt kit reagents should be stored at 4°C, except the ATP must be stored at -20°C. Coated assay plates should be stored in the original foil bag sealed by the zip lock and containing a desiccant pack.

For research use only, not for use in diagnostic or therapeutic procedures

Sample Preparation

Numerous extraction and purification methods can be used to isolate Cdc2-Cyclin B. The following protocols have been shown to work with a number of different tissues and enzyme sources, and are provided as examples of suitable methods. Highly purified Cdc2-Cyclin B should be diluted. It is strongly advised that the user always perform an initial experiment to determine the proper dilution to be used in subsequent experiments. This need not be any more than a single time point assay using serial dilutions of the sample fraction taken prior to a purification step. One eight well strip of the substrate plate should be sufficient for this initial experiment. All sample preparation should be performed at 4°C and recovered fractions be kept at 4°C to prevent loss of enzymatic activity.

CAUSION: It should be noted that this assay kit might detect other protein kinases, in addition to Cdc2-Cyclin B kinase, in crude extract and column sample. You should trace Cyclin B protein level by western blotting in column fractions.

Preparation of Cell Lysates

1. Grow cells on 10 cm dish at 80 % confluent overnight.

2. Add appropriate amount of microtubuli-destabilizing drug (e.g. final 0.25 µM Nocodazol) and culture for appropriate period (e.g. final 0.25 µM Nocodazol, culture for 16 hrs).

3. Shake off the cells and harvest by centrifugation using standard methods.

4. Resuspend the cell pellet with 500 µL of an appropriate extraction buffer (for example; 20 mM Tris-HCl, pH 8.5, 250 mM NaCl, 1 mM DTT, 1 mM EDTA, 1 mM EGTA, 0.2 mM PMSF, 1 µg/mL pepstatin, 0.5 µg/mL leupeptin, 5 mM beta-glycerophosphate, 5 mM NaF, 1 mM Na_3VO_4, 5 mM mercaptoethanol) and lyse the resuspended cells using either a Dounce Homogenizer, sonication, or three cycles of freezing and thawing.

5. Transfer extracts to microcentrifuge tubes and centrifuge at 15,000 rpm for 5 minutes.

6. Dilute cell extracts 1:5 with Q-buffer (20 mM Tris-HCl, pH 8.5, 0.2 mM EDTA, 1 mM EGTA, 1 µg/mL pepstatin, 0.5 µg/mL leupeptin, 0.2 mM Na_3VO_4, 5 mM mercaptoethanol). Stand on ice for 15 min.

7. Centrifuge diluted extracts at 15,000 rpm for 15 minutes. Take a supernatant.

Column Purification Fractions

8. Apply the supernatant to a column of Q sepharose column (gel volume: 2 ml, Amersham Pharmacia Biosytems) equilibrated with Q-buffer.
9. Wash the column with five column volumes of Q-buffer.

10. Elute the column with a linear gradient of NaCl (0.05-0.5 M) in Q-buffer collecting 1-2 mL fractions. These samples are now ready for analysis according to the instructions provided in **Detailed Protocol**.

NOTE: THE ABOVE PROCEDURES ARE INTENDED ONLY AS A GUIDELINE. THE OPTIMAL EXPERIMENTAL CONDITIONS WILL VARY DEPENDING ON THE

PARAMETERS BEING INVESTIGATED, AND MUST BE DETERMINED BY THE INDIVIDUAL USER. NO WARRANTY OR GUARANTEE OF PERFORMANCE USING THESE PROCEDURES IS MADE OR IMPLIED.

Example of Test Results

Fig.1 Dose dependency of recombinant Cdc2-Cyclin B enzyme reaction

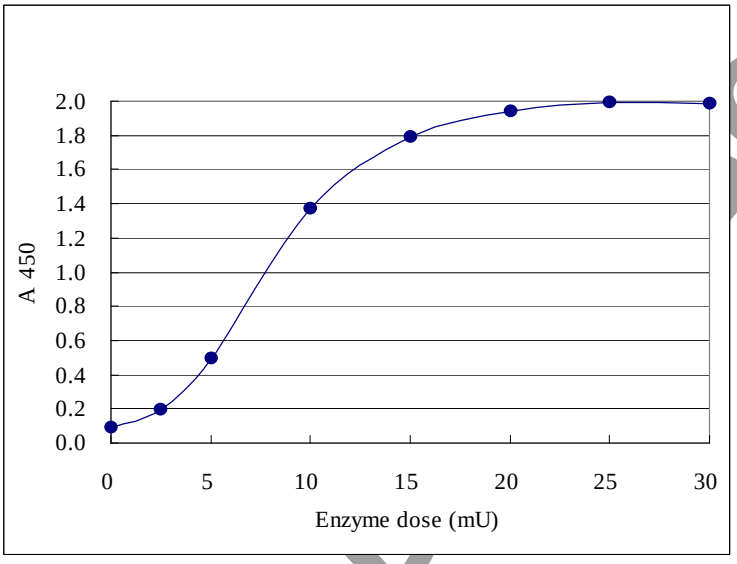


Fig.2 Time course of recombinant Cdc2-Cyclin B enzyme reaction

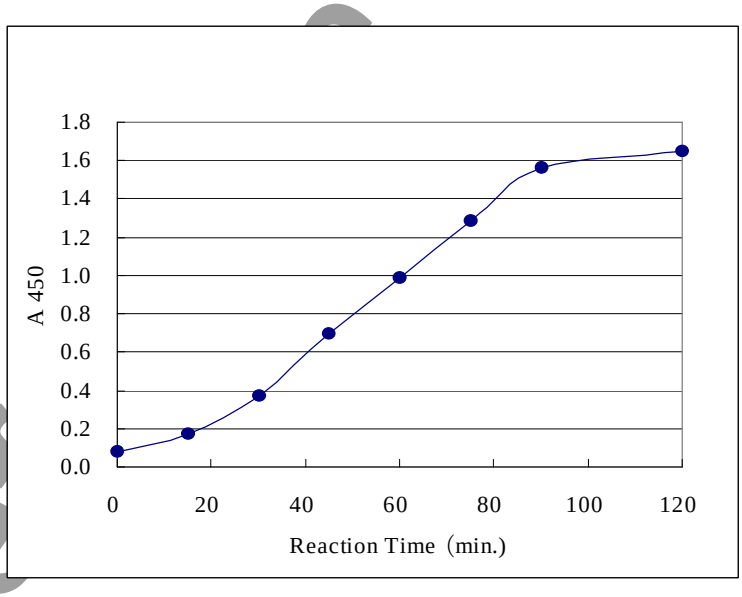


Fig.3 Km for ATP (recombinant Cdc2-Cyclin B)

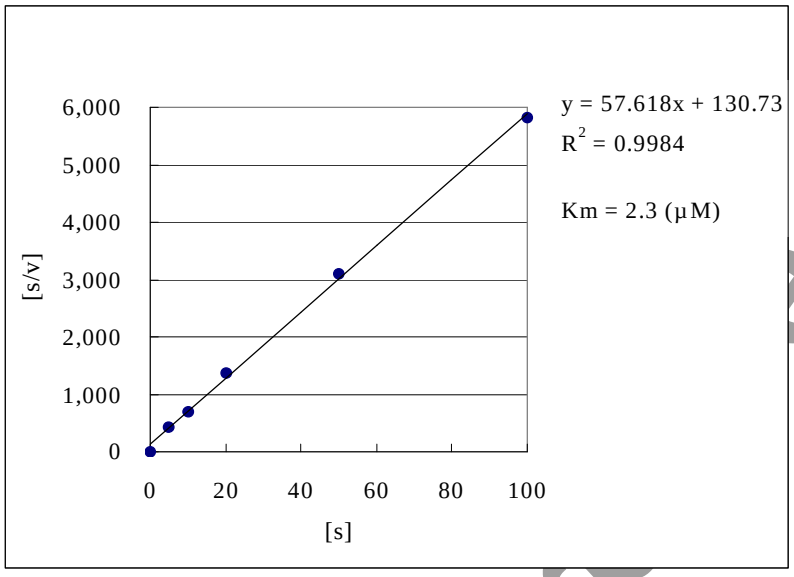
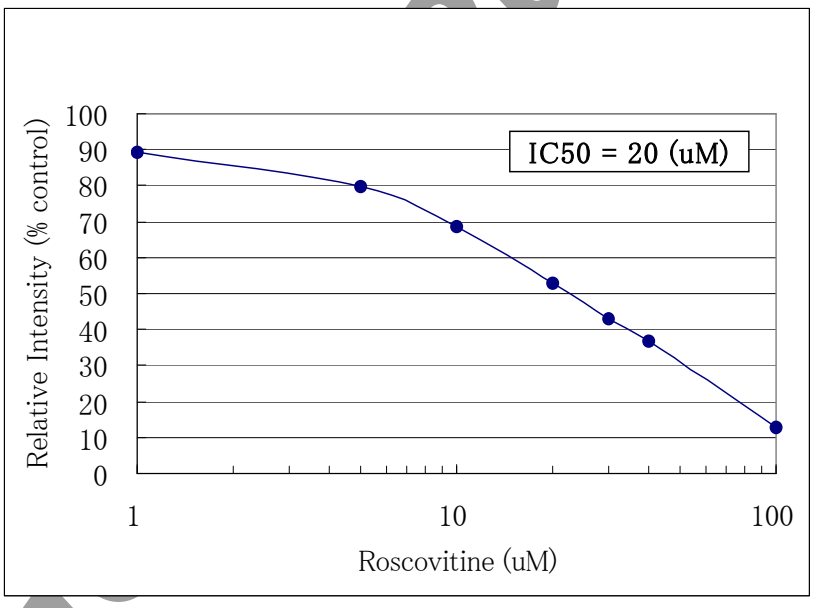


Fig.4 Effect of cdk-specific inhibitor Roscovitine on Cdc2-Cyclin B activity



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Related Products

*** Cdc2-Cyclin B Positive control: Cat# CY-E1164**
*** Anti-Phospho-Cdc7-Thr376 Monoclonal Antibody (TK-3H7): Cat# CY-M1021**

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