Product Manual

Checkpoint Kinase Activity Immunoblot Kit

Catalog Number

STA- 413 **20 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Cdc25C is a protein phosphatase responsible for dephosphorylating and activating Cdc2, a critical step in regulating mitosis in eukaryotic cells. Cdc25C is constitutively phosphorylated at Ser^{216} throughout interphase by c-TAK1 (Cdc25C associated protein kinase), while phosphorylation at this site is DNA damage dependent at the G2/M checkpoint. Prior to mitosis, checkpoint kinases (CHK1 and CHK2) can be activated in response to DNA damage, phosphorylating Cdc25C at Ser^{216}, ultimately leading to cell cycle arrest. Consequently, a cell is prevented from entering mitosis and passing this damage to daughter cells. Mutations to these checkpoint kinases lead to decreased DNA repair and increased susceptibility to cancer.

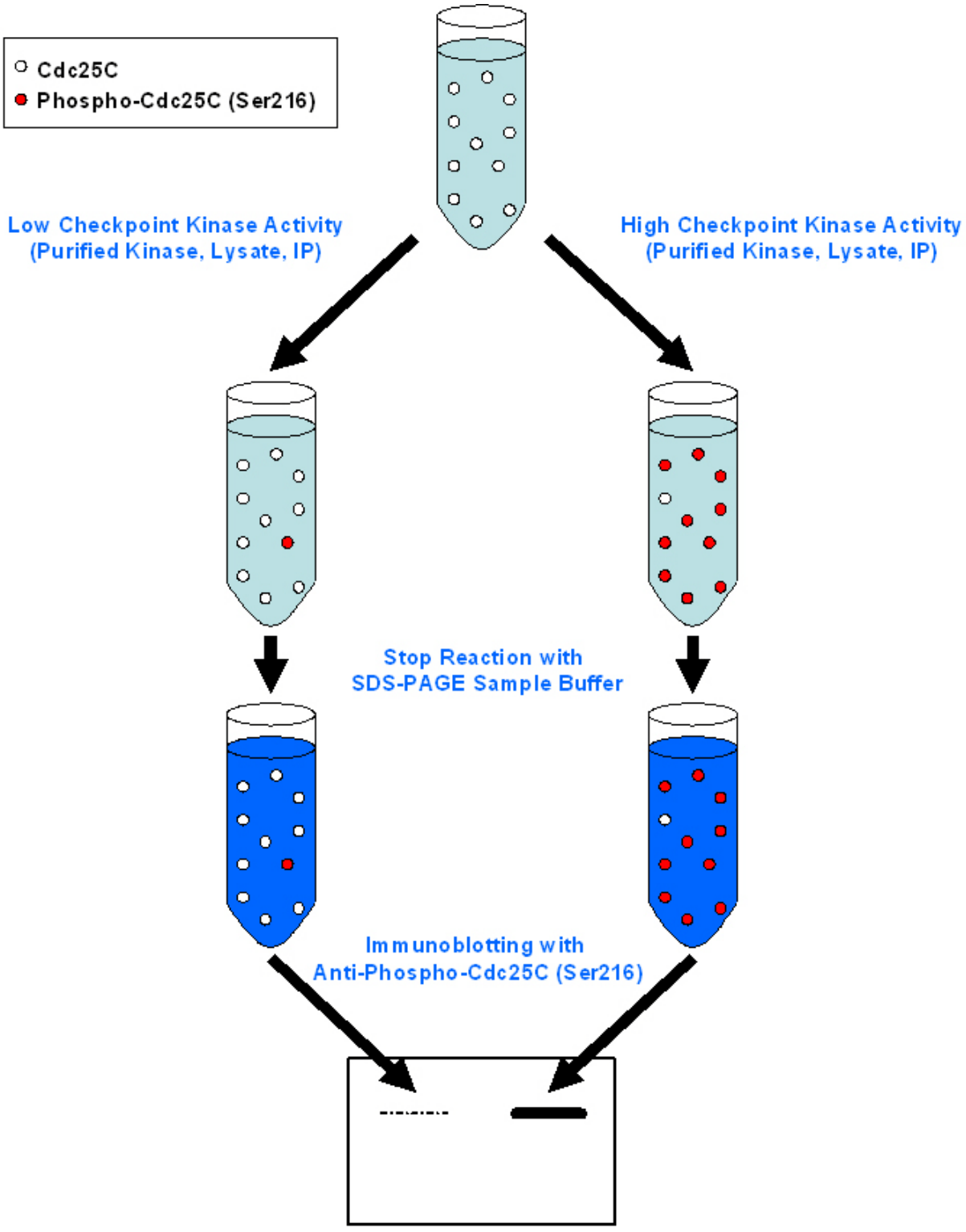
Cell Biolabs' Checkpoint Kinase Activity Immunoblot Kit utilizes recombinant Cdc25C as checkpoint kinase substrate. After incubating the substrate with checkpoint kinase samples (such as purified kinase, cell lysate or immunoprecipitate), the phosphorylated Cdc25C is detected by western blot analysis using an anti-phospho-Cdc25C (Ser^{216}) antibody (Figure 1).

Cell Biolabs' Checkpoint Kinase Activity Immunoblot Kit provides a simple and fast tool to monitor checkpoint kinase activity using its physiological substrate. The kit also includes active CHK1 as a positive control. Each kit provides sufficient quantities to perform 20 assays.

Related Products

1. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
2. STA-321: OxiSelect™ DNA Double-Strand Break (DSB) Staining Kit
3. STA-322: OxiSelect™ UV-induced DNA Damage ELISA Kit (CPD Quantitation)
4. STA-323: OxiSelect™ UV-induced DNA Damage ELISA Kit (6-4PP Quantitation)
5. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP sites)
6. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
7. STA-326: OxiSelect™ Cellular UV-induced DNA Damage ELISA Kit (CPD)
8. STA-327: OxiSelect™ Cellular UV-induced DNA Damage Staining Kit (CPD)
9. STA-328: OxiSelect™ Cellular UV-induced DNA Damage ELISA Kit (6-4PP)
10. STA-329: OxiSelect™ Cellular UV-induced DNA Damage Staining Kit (6-4PP)
11. STA-415: ROCK Activity Immunoblot Kit
12. STA-416: 96-well ROCK Activity Assay Kit

Assay Principle



Kit Components

1. Checkpoint Kinase Substrate (Part No 241301): One vial – 40 µL containing 4 mg/mL recombinant Cdc25C.

2. 10X Kinase Buffer (Part No. 241502): Three vials – 1.0 mL each of 250 mM Tris, pH 7.5, 100 mM $MgCl_2$, 50 mM Glycerol-2-Phosphate, 1 mM Na_3VO_4.

3. ATP Solution (Part No. 241503): One vial – 400 µL of 10 mM ATP.

4. Anti-phospho-Cdc25 (Ser^{216}) (Part No. 241302): One vial – 50 µL.

5. Secondary Antibody, HRP-conjugate (Part No. 230805): One 100 µL vial

6. Active CHK1 (Part No. 241303): One vial – 20 µL containing 50 ng active CHK1 in 25 mM Tris, pH 7.5, 10 mM $MgCl_2$, 5 mM Glycerol-2-Phosphate, 0.1 mM Na_3VO_4, 10% Glycerol, 0.1% BSA.

Materials Not Supplied

1. Checkpoint kinase sample (purified kinase, cell lysate or immunoprecipitate)
2. Lysis Buffer: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM 2-glycerophosphate, 1 % Triton X-100 or 1 % Nonidet P-40, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4 and Proteinase inhibitors.
3. DTT
4. 30°C incubator or water bath
5. 4X SDS-PAGE sample buffer
6. Electrophoresis and immunoblotting systems
7. Immunoblotting wash buffer such as TBST (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween-20)
8. Immunoblotting blocking buffer (TBST containing 5% Non-fat Dry Milk)
9. PVDF or nitrocellulose membrane
10. ECL Detection Reagents

Storage

Store active CHK1 at -80ºC and all other kit components at -20ºC. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Kinase Buffer: Dilute to 10X Kinase Buffer to 1X in deionized water. 1X Kinase Buffer may be stored at 4ºC for short term (1-2 weeks). Just prior to usage, add DTT to a final concentration of 1 mM.

- 1X Kinase/ATP/Substrate Solution: For each kinase assay, freshly prepare 50 µL of 1X Kinase/ATP/Substrate Solution by adding 1 µL of 10 mM ATP solution, 2 µL of Checkpoint Kinase Substrate to 47 µL of 1X Kinase Buffer containing DTT.

Assay Protocol

I. Kinase Reaction

1a. For immunoprecipitations with anti-checkpoint kinase antibody: Kinase is first immunoprecipitated from cell or tissue lysate with an anti-checkpoint kinase antibody and Protein A/G bead slurry. Immediately before the kinase assay, wash bead slurry once with 1X Kinase Buffer, remove all supernatant, and assay immediately by adding 50 µL of 1X Kinase/ATP/Substrate Solution directly to the beads and mixing well.

1b. Purified Kinase or Cell Lysate: Purified kinase or cell lysate sample can be used directly in the kinase assay or further diluted with 1X Kinase Buffer. Add 25 µL of checkpoint kinase sample to a microcentrifuge tube, and initiate the kinase reaction by adding 50 µL of 1X Kinase/ATP/ Substrate Solution.

2. (optional) Add 4 µL of the provided active CHK1 and 21 µL of 1X Kinase Buffer to a microcentrifuge tube; initiate the kinase reaction by adding 50 µL of 1X Kinase/ATP/Substrate Solution.
3. Incubate the tubes at 30°C for 30-60 minutes with gentle agitation.
4. Stop kinase reaction by adding 25 µL of 4X reducing SDS-PAGE sample buffer.
5. Boil each sample for 5 minutes.
6. Centrifuge each sample for 10 seconds at 12,000 x g.

II. Electrophoresis and Transfer

1. Load 20 µL of supernatant to a polyacrylamide gel. Also, it's recommended to include a pre-stained MW standard (as an indicator of a successful transfer in step 3).
2. Perform SDS-PAGE as per the manufacturer's instructions.
3. Transfer the gel proteins to a PVDF or nitrocellulose membrane as per the manufacturer's instructions.

III. Immunoblotting and Detection (all steps are at room temperature, with agitation)

1. Following the electroblotting step, immerse the PVDF membrane in 100% Methanol for 15 seconds, and then allow it to dry at room temperature for 5 minutes.

   Note: If Nitrocellulose is used instead of PVDF, this step should be skipped.
2. Block the membrane with 5% non-fat dry milk in TBST for 1 hr at room temperature with constant agitation.
3. Incubate the membrane with the anti-phospho-Cdc25C (Ser^{216}) antibody, freshly diluted 1:1000 in 5% non-fat dry milk/TBST, for 2 hr at room temperature with constant agitation.

   Note: To conserve antibody, incubations should be performed in a plastic bag.
4. Wash the blotted membrane three times with TBST, 5 minutes each time.
5. Incubate the membrane with the secondary antibody, HRP-conjugated, freshly diluted in 1:1000 in 5% non-fat dry milk/TBST, for 1 hr at room temperature with constant agitation.

6. Wash the blotted membrane three times with TBST, 5 minutes each time.
7. Use the detection method of your choice. We recommend enhanced chemiluminescence reagents from Pierce.

Example of Results

The following figure demonstrates typical results seen with Cell Biolabs' Checkpoint Kinase Activity Immunoblot Kit. One should use the data below for reference only.

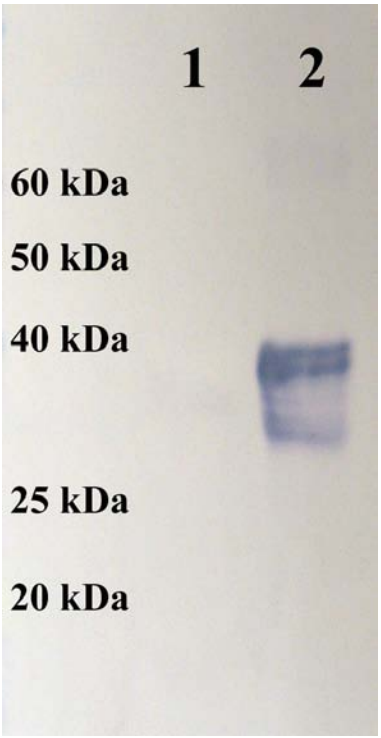


Figure 1: CHK1 Activity Immunoblot Assay. 25 µL of 1X Kinase Buffer containing 10 ng of active CHK1 was incubated with 50 µL of 1X Kinase Buffer containing 0.2 mM ATP and 8 µg of recombinant Cdc25C for 60 minutes at 30°C. Kinase reaction was stopped by adding 25 µL of 4X SDS-PAGE Sample Buffer. **Lane 1**: Without kinase (negative control); **Lane 2**: with kinase. Phosphorylation of Cdc25C substrate was detected by anti-phospho-Cdc25C (Ser^{216}) antibody as described in Assay Protocol.

References

1. Zhou, B. B., and Elledge, S. J. (2000) *Nature* **408**, 433-439
2. Matsuoka, S., Huang, M., and Elledge, S. J. (1998) *Science* **282**, 1893-1897
3. Ahn, J., Urist, M., and Prives, C. (2004) *DNA Repair* **3**, 1039-1047
4. Ahn, J., and Prives, C. (2002) *J. Biol. Chem.* **277**, 48418-48426

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