Store at 4°C

#7184

PathScan® Phospho-cdc2 (Thr161) Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

New 06/06

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Species Cross-Reactivity: H

Introduction: CST's PathScan® Phospho-cdc2 (Thr161) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Phospho-cdc2 (Thr161) protein. A Phospho-cdc2 (Thr161) Rabbit polyclonal Ab #9114* has been coated onto the microwells. After incubation with cell lysates, phospho-cdc2 (Thr161) protein is captured by the coated antibody. Following extensive washing, cdc2 Mouse mAb #2658* is added to detect the captured phospho-cdc2 protein. Anti-mouse IgG, HRP-linked Antibody #7076* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of phospho-cdc2 (Thr161) protein.

*Antibodies in this kit are custom formulations specific to this kit.

Companion Products:

Anti-mouse IgG, HRP-linked Antibody #7076

Pathscan® Phospho-cdc2 (Tyr15) Sandwich ELISA Kit #7176

Phospho-cdc2 (Thr161) Antibody #9114

Specificity/Sensitivity: CST's PathScan® Phospho-cdc2 (Thr161) Sandwich ELISA Kit detects endogenous levels of phospho-cdc2 (Thr161). As shown in Figure 1, using the Phospho-cdc2 (Thr161) ELISA Kit #7184, a significant induction of Phospho-cdc2 (Thr161) is detected in HeLa cells treated with Interleukin-4.

Products Included	Volume	Solution Color
cdc2 (Thr161) Ab Coated Microwells*	96 tests	
cdc2 Detection Antibody	11 ml	green
Anti-mouse IgG HRP-Linked Ab	11 ml	red
TMB Substrate	11 ml	colorless
STOP Solution	11 ml	colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	colorless
Sample Diluent	25 ml	blue
10X Cell Lysis Buffer #9803**	15 ml	yellowish

* 12 8-well modules -Each module is designed to break apart for 8 tests.
**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at –20°C (packaged separately).

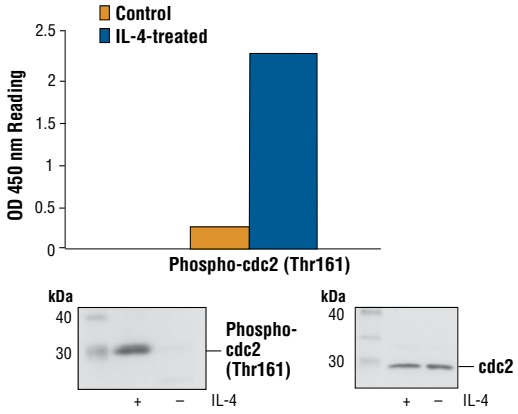

Figure 1: Treatment of HeLa cells with Interleukin-4 stimulates phosphorylation of cdc2 at Thr161 as detected by PathScan® Phospho-cdc2 (Thr161) Sandwich ELISA kit #7184, but does not affect the level of total cdc2 protein detected using cdc2 Mouse mAb (#9116) via Western analysis. OD 450 readings are shown in the top figure, while the corresponding Western blot using Phospho-cdc2 (Thr161) Antibody #9114 (left panel) or cdc2 Mouse mAb #9116 (right panel), is shown in the bottom figure.

© 2006 Cell Signaling Technology, Inc.

Background: Entry of all eukaryotic cells into mitosis is regulated by activation of cdc2 kinase. Activation of cdc2 is controlled at several steps including cyclin binding and phosphorylation of Thr161 (1-4). However, the critical regulatory step in activating cdc2 during progression into mitosis appears to be dephosphorylation of Tyr15 and Thr14 (3,5). Phosphorylation at Tyr15 and inhibition of cdc2 is carried out by Wee1 and Myt1 protein kinases, while Tyr15 dephosphorylation and activation of cdc2 is carried out by the cdc25 phosphatase (3,4,6).

Background References:

(1) Norbury, C. and Nurse, P. (1992) *Annu. Rev. Biochem.* 61, 441–470.

(2) Atherton-Fessler, S. et al. (1993) *Mol. Cell. Biol.* 13, 1675–1685.

(3) Watanabe, N. et al. (1995) *EMBO J.* 14, 1878–1891.

(4) Galaktionov, K. et al. (1995) *Genes Dev.* 9, 1046–1058.

(5) Hunter, T. (1995) *Cell* 80, 225–236.

(6) McGowan, C.H. and Russell, P. (1993) *EMBO J.* 12, 75–85.

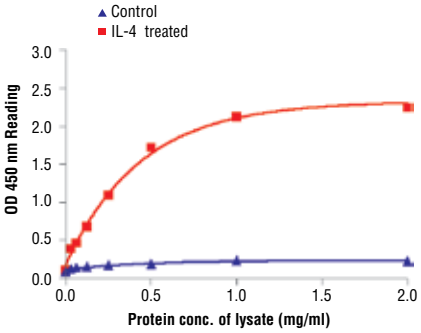

Figure 2. The relationship between protein concentration of lysates from untreated and Interleukin-4 treated HeLa cells and kit assay optical density reading. HeLa cells (80% confluent) were treated with human Interleukin-4 for 10 minutes (100 ng/ml).

© 2006 Cell Signaling Technology, Inc.

Sandwich ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X wash buffer (included in each Pathscan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #9803:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1µg/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm^2) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Add 100 µl of Sample Diluent (supplied in each Pathscan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 µl of cell lysate into the tube and vortex for a few seconds. (Sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction.) Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
3. Add 100 µl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.
4. Gently remove the tape and wash wells:
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.
   c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   d. Clean the underside of all wells with a lint-free tissue.
5. Add 100 µl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step C4.
7. Add 100 µl of HRP-Linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.
8. Repeat wash procedure as in Step C4.
9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes at 25°C.
10. Add 100 µl of STOP Solution to each well. Shake gently for a few seconds.

NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

11. Read results.
    a. Visual Determination — Read within 30 minutes after adding STOP Solution.
    b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.

© 2005 Cell Signaling Technology, Inc.