



#7870 Store at +4C

PathScan® Phospho-Chk1 (Ser317) Sandwich ELISA Kit

1 Kit (96 assays)

Species Cross Reactivity: H UniProt ID: #O14757 Entrez-Gene Id: #1111

 Orders: 877-616-CELL (2355)
orders@cellsignal.com

Support: 877-678-TECH (8324)

 Web: info@cellsignal.com
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Quantity	Color	Storage Temp
Chk1 Mouse mAb Coated Microwells	19914	96 tests		+4C
Phospho-Chk1 (Ser317) Rabbit Detection Antibody	14014	1 ea	Green (Lyophilized)	+4C
Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated)	13272	1 ea	Red (Lyophilized)	+4C
Detection Antibody Diluent	13339	11 ml	Green	+4C
HRP Diluent	13515	11 ml	Red	+4C
TMB Substrate	7004	11 ml		+4C
STOP Solution	7002	11 ml		+4C
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml		+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Cell Lysis Buffer (10X)	9803	15 ml		-20C

Kit contents scale proportionally with size, except sealing tape.

Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

The PathScan® Phospho-Chk1 (Ser317) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Chk1 when phosphorylated at Ser317. An Chk1 mouse antibody has been coated onto the microwells. After incubation with cell lysates, Chk1 (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a phospho-Chk1 (Ser317) rabbit detection antibody is added to detect phosphorylation of Ser317 on the captured Chk1 protein. Anti-rabbit IgG, HRP-linked antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of Chk1 phosphorylated at Ser317.

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

Specificity/Sensitivity

CST's PathScan® Phospho-Chk1 (Ser317) Sandwich ELISA Kit #7870 detects endogenous levels of Chk1 protein when phosphorylated at Ser317. As shown in Figure 1, a significant induction of Chk1 phosphorylation at Ser317 can be detected in HeLa cells following treatment with UV using the Phospho-Chk1 (Ser317) Sandwich ELISA Kit #7870. The levels of total Chk1 (phospho and nonphospho) remain unchanged as shown by Western analysis and by PathScan® Total Chk1 Sandwich ELISA Kit #7872 (Figure 1). This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background

Chk1 kinase acts downstream of ATM/ATR kinase and plays an important role in DNA damage checkpoint control, embryonic development, and tumor suppression (1). Activation of Chk1 involves phosphorylation at Ser317 and Ser345 by ATM/ATR, followed by autophosphorylation of Ser296. Activation occurs in response to blocked DNA replication and certain forms of genotoxic stress (2). While phosphorylation at Ser345 serves to localize Chk1 to the nucleus following checkpoint activation (3), phosphorylation at Ser317 along with site-specific phosphorylation of PTEN allows for re-entry into the cell cycle following stalled DNA replication (4). Chk1 exerts its checkpoint mechanism on the cell cycle, in part, by regulating the cdc25 family of phosphatases. Chk1 phosphorylation of cdc25A targets it for proteolysis and inhibits its activity through 14-3-3 binding (5). Activated Chk1 can inactivate cdc25C via phosphorylation at Ser216, blocking the activation of cdc2 and transition into mitosis (6). Centrosomal Chk1 has been shown to phosphorylate cdc25B and inhibit its activation of CDK1-cyclin B1, thereby abrogating mitotic spindle formation and chromatin condensation (7). Furthermore, Chk1 plays a role in spindle checkpoint function through regulation of aurora B and BubR1 (8). Research

studies have implicated Chk1 as a drug target for cancer therapy as its inhibition leads to cell death in many cancer cell lines (9).

Background References

1. Liu, Q. et al. (2000) *Genes Dev* 14, 1448-59.
2. Zhao, H. and Piwnica-Worms, H. (2001) *Mol Cell Biol* 21, 4129-39.
3. Jiang, K. et al. (2003) *J Biol Chem* 278, 25207-17.
4. Martin, S.A. and Ouchi, T. (2008) *Mol Cancer Ther* 7, 2509-16.
5. Chen, M.S. et al. (2003) *Mol Cell Biol* 23, 7488-97.
6. Zeng, Y. et al. (1998) *Nature* 395, 507-10.
7. Löffler, H. et al. (2006) *Cell Cycle* 5, 2543-7.
8. Zachos, G. et al. (2007) *Dev Cell* 12, 247-60.
9. Garber, K. (2005) *J Natl Cancer Inst* 97, 1026-8.

Trademarks and Patents

Cell Signaling Technology is a trademark of Cell Signaling Technology, Inc.

PathScan is a registered trademark of Cell Signaling Technology, Inc.

All other trademarks are the property of their respective owners. Visit cellsignal.com/trademarks for more information.

Limited Uses

Except as otherwise expressly agreed in a writing signed by a legally authorized representative of CST, the following terms apply to Products provided by CST, its affiliates or its distributors. Any Customer's terms and conditions that are in addition to, or different from, those contained herein, unless separately accepted in writing by a legally authorized representative of CST, are rejected and are of no force or effect.

Products are labeled with For Research Use Only or a similar labeling statement and have not been approved, cleared, or licensed by the FDA or other regulatory foreign or domestic entity, for any purpose. Customer shall not use any Product for any diagnostic or therapeutic purpose, or otherwise in any manner that conflicts with its labeling statement. Products sold or licensed by CST are provided for Customer as the end-user and solely for research and development uses. Any use of Product for diagnostic, prophylactic or therapeutic purposes, or any purchase of Product for resale (alone or as a component) or other commercial purpose, requires a separate license from CST. Customer shall (a) not sell, license, loan, donate or otherwise transfer or make available any Product to any third party, whether alone or in combination with other materials, or use the Products to manufacture any commercial products, (b) not copy, modify, reverse engineer, decompile, disassemble or otherwise attempt to discover the underlying structure or technology of the Products, or use the Products for the purpose of developing any products or services that would compete with CST products or services, (c) not alter or remove from the Products any trademarks, trade names, logos, patent or copyright notices or markings, (d) use the Products solely in accordance with CST Product Terms of Sale and any applicable documentation, and (e) comply with any license, terms of service or similar agreement with respect to any third party products or services used by Customer in connection with the Products.

#7870

PathScan[®] Phospho-Chk1 (Ser317) Sandwich ELISA Kit

ELISA Colorimetric (Lyophilized)

A. Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
3. **HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody.
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody.
6. **Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan[®] Sandwich ELISA Kit) in purified water.
8. **Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803; This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) immediately before use.
9. **TMB Substrate** (#7004).
10. **STOP Solution** (#7002).

***NOTE:** Some PathScan[®] ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.

B. Preparing Cell Lysates

For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 rpm) 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.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) 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. Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan[®] Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.

4. Gently remove the tape and wash wells:
 1. Discard plate contents into a receptacle.
 2. Wash 4 times with 1X Wash Buffer, 200 μ l each time for each well.
 3. 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.
 4. Clean the underside of all wells with a lint-free tissue.
5. Add 100 μ l of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 μ l of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 μ l of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
10. Add 100 μ l 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.

1. Visual Determination: Read within 30 min after adding STOP Solution.
2. Spectrophotometric Determination: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.

posted November 2013