Revision 4

Store at +4C

#60174

PathScan® RP Phospho-PLK1 (Thr210) Sandwich ELISA Kit

Cell Signaling TECHNOLOGY®

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1 Kit (96 assays)

Species Cross Reactivity: H
UniProt ID: #P53350
Entrez-Gene Id: #5347

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

Product Includes	Product #	Quantity	Color	Storage Temp
Phospho-PLK1 (Thr210) Rabbit mAb Coated Microwells	77588	96 tests		+4C
PLK1 Rabbit Detection mAb	17378	1 ea	Red (Lyophilized)	+4C
HRP Diluent	13515	5.5 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
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 rapid protocol (RP) PathScan® RP Phospho-PLK1 (Thr210) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of PLK1 protein phosphorylated at Thr210 in a reduced assay time of 1.5 hours. Incubation of cell lysates and detection antibody on the coated microwell plate forms a sandwich with phospho-PLK1 (Thr210) in a single step. The plate is then extensively washed and TMB reagent is added for signal development. The magnitude of absorbance for the developed color is proportional to the quantity of phospho-PLK1 (Thr210). Learn more about all of your ELISA kit options here.

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

Specificity/Sensitivity

The PathScan® RP Phospho-PLK1 (Thr210) Sandwich ELISA Kit detects endogenous levels of PLK1 protein phosphorylated at Thr210. The kit sensitivity is shown in 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

At least four distinct polo-like kinases exist in mammalian cells: PLK1, PLK2, PLK3, and PLK4/SAK (1). PLK1 apparently plays many roles during mitosis, particularly in regulating mitotic entry and exit. The mitosis promoting factor (MPF), cdc2/cyclin B1, is activated by dephosphorylation of cdc2 (Thr14/Tyr15) by cdc25C. PLK1 phosphorylates cdc25C at Ser198 and cyclin B1 at Ser133, causing translocation of these proteins from the cytoplasm to the nucleus (2-5). PLK1 phosphorylation of Myt1 at Ser426 and Thr495 has been proposed to inactivate Myt1, one of the kinases known to phosphorylate cdc2 at Thr14/Tyr15 (6). Polo-like kinases also phosphorylate the cohesin subunit SCC1, causing cohesin displacement from chromosome arms that allow for proper cohesin localization to centromeres (7). Mitotic exit requires activation of the anaphase promoting complex (APC) (8), a ubiquitin ligase responsible for removal of cohesin at centromeres, and degradation of securin, cyclin A, cyclin B1, Aurora A, and cdc20 (9). PLK1 phosphorylation of the APC subunits Apc1, cdc16, and cdc27 has been demonstrated *in vitro* and has been proposed as a mechanism by which mitotic exit is regulated (10,11).

Substitution of Thr210 with Asp has been reported to elevate PLK1 kinase activity and delay/arrest cells in mitosis, while a Ser137Asp substitution leads to S-phase arrest (12). In addition, while DNA damage has been found to inhibit PLK1 kinase activity, the Thr210Asp mutant is resistant to this inhibition (13). PLK1 has been reported to be phosphorylated *in vivo* at Ser137 and Thr210 in mitosis; DNA damage prevents phosphorylation at these sites (14).

Background References

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4. Peter, M. et al. (2002) *EMBO Rep* 3, 551-6.
5. Jackman, M. et al. (2003) *Nat Cell Biol* 5, 143-8.
6. Nakajima, H. et al. (2003) *J Biol Chem* 278, 25277-80.
7. Sumara, I. et al. (2002) *Mol Cell* 9, 515-25.
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13. Smits, V.A. et al. (2000) *Nat Cell Biol* 2, 672-6.
14. Tsvetkov, L. and Stern, D.F. (2005) *Cell Cycle* 4, 166-71.

Trademarks and Patents

Limited Uses

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PathScan® RP Phospho-PLK1 (Thr210) Sandwich ELISA Kit

PathScan® Sandwich ELISA Protocol (Rapid Protocol)

NOTE: This protocol is for PathScan® kits that use an HRP directly conjugated to the detection antibody **(Rapid Protocol)**, rather than a 2-step method where the detection antibody and a secondary-HRP are added sequentially.

A. Solutions and Reagents

NOTE: Prepare solutions with deionized/purified water or equivalent.

1. **Microwell strips:** Bring all to room temperature before opening bag/use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack and stored at 4°C.
2. **Detection Antibody:** Reconstitute lyophilized Detection Antibody (red colored cake) with 1 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 full 1 mL of reconstituted Detection Antibody to 4.5 mL of HRP Diluent in a clean tube and gently mix. For best results, use immediately following antibody reconstitution. Unused reconstituted Detection Antibody may be stored for up to 4 weeks at 4°C, although there may be some loss of signal compared to freshly reconstituted antibody.
3. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the Detection Antibody that is linked to HRP.
4. **1X ELISA Wash Buffer:** Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) to 1X with deionized water.
5. **1X Cell Lysis Buffer:** Prepare by diluting 10X Cell Lysis Buffer #9803 to 1X with deionized water. This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: When using to prepare cell lysates, add Protease/Phosphatase Inhibitor Cocktail (#5872, not supplied) and 1 mM phenylmethyl- sulfonyl fluoride (PMSF, #8553, not supplied) immediately before use.
6. **TMB Substrate (#7004):** Bring to room temperature before use.
7. **STOP Solution (#7002):** Bring to room temperature before use.

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 including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail 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 (14,000 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 10^6 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 including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 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

NOTE: Equilibrate all materials and prepared reagents to room temperature prior to running the assay.

1. Prepare all reagents as indicated above (Section A).
2. Samples should be undiluted or diluted with 1X Cell Lysis Buffer to a 2X protein concentration in order to achieve a final 1X protein concentration upon addition of the Detection Antibody. 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 results across a range of lysate concentration points.
3. Add 50 µL of each sample to the appropriate wells.
4. Add 50 µL of the Detection Antibody to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm (moderate agitation).

6. Gently remove the tape and wash wells:
    1. Discard plate contents into a receptacle.
    2. Wash 4 times with 1X Wash Buffer, 200 µL 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.
7. Add 100 µL of TMB Substrate to each well. Seal with tape and incubate the plate in the dark for 15 min at room temperature on a plate shaker (400 rpm, moderate agitation) or alternatively for 10 min at 37°C without shaking.
8. 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.

9. 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.

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