Revision 1

Store at +4C

#7795

PathScan® Phospho-Vimentin (Ser56) Sandwich ELISA Kit

Cell Signaling TECHNOLOGY®

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Species Cross Reactivity: H

UniProt ID: #P08670

Entrez-Gene Id: #7431

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

Product Includes	Product #	Quantity	Color	Storage Temp
TMB Substrate	7004	11 ml	Colorless	+4C
STOP Solution	7002	11 ml	Colorless	+4C
Sealing Tape	54503	2 ea		+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-20C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	+4C

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-Vimentin (Ser56) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of vimentin protein when phosphorylated at Ser56. A Phospho-Vimentin (Ser56) Rabbit Antibody has been coated onto the microwells. After incubation with cell lysates, phospho-vimentin protein is captured by the coated antibody. Following extensive washing, a Vimentin Mouse Detection Antibody is added to detect the captured phospho-vimentin protein. Anti-mouse IgG, HRP-linked antibody 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 vimentin protein.
Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity

The PathScan® Phospho-Vimentin (Ser56) Sandwich ELISA Kit detects endogenous levels of vimentin protein when phosphorylated at Ser56 as shown in Figure 1. Kit sensitivity is shown in Figure 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background

The cytoskeleton consists of three types of cytosolic fibers: microfilaments (actin filaments), intermediate filaments, and microtubules. Major types of intermediate filaments are distinguished by their cell-specific expression: cytokeratins (epithelial cells), glial fibrillary acidic protein (GFAP) (glial cells), desmin (skeletal, visceral, and certain vascular smooth muscle cells), vimentin (mesenchyme origin), and neurofilaments (neurons). GFAP and vimentin form intermediate filaments in astroglial cells and modulate their motility and shape (1). In particular, vimentin filaments are present at early developmental stages, while GFAP filaments are characteristic of differentiated and mature brain astrocytes. Thus, GFAP is commonly used as a marker for intracranial and intraspinal tumors arising from astrocytes (2). Research studies have shown that vimentin is present in sarcomas, but not carcinomas, and its expression is examined in conjunction with that of other markers to distinguish between the two (3). Vimentin's dynamic structural changes and spatial re-organization in response to extracellular stimuli help to coordinate various signaling pathways (4). Phosphorylation of vimentin at Ser56 in smooth muscle cells regulates the structural arrangement of vimentin filaments in response to serotonin (5,6). Remodeling of vimentin and other intermediate filaments is important during lymphocyte adhesion and migration through the endothelium (7).
During mitosis, CDK1 phosphorylates vimentin at Ser56. This phosphorylation provides a PLK binding site for vimentin-PLK interaction. PLK further phosphorylates vimentin at Ser83, which might serve as memory phosphorylation site and play a regulatory role in vimentin filament disassembly (8,9). Additionally, studies using various soft-tissue sarcoma cells have shown that phosphorylation of vimentin at Ser39 by Akt1 enhances cell migration and survival, suggesting that vimentin could be a potential target for soft-tissue sarcoma targeted therapy (10,11).

Background References

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3. Leader, M. et al. (1987) *Histopathology* 11, 63-72.

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6. Fomina, I.G. et al. (1990) *Klin Med (Mosk)* 68, 125-7.
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Trademarks and Patents

Limited Uses

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ELISA Colorimetric

NOTE: Refer to product-specific datasheets or product webpage for assay incubation temperature.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS)**: (#9808) To prepare 1 L PBS: add 50 ml 10X PBS to 950 ml dH_2O, mix.
2. Bring all microwell strips to room temperature before use.
3. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in dH_2O.
4. **1X Cell Lysis Buffer**: 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH_2O, mix. Buffer can be stored at 4°C for short-term use (1–2 weeks).

   Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

   NOTE: Refer to product-specific datasheet or webpage for lysis buffer recommendation.

5. **TMB Substrate**: (#7004).
6. **STOP Solution**: (#7002).

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 (~1,200 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 (~1,200 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 in the storage bag 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 or product webpage for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
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 µl each time per well.
    3. For each wash, strike plates on fresh paper 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 µl of detection antibody (green color) to each well. Seal with tape and incubate the plate at 37°C for 1 hr.

6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl 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 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**
    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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