Revision 1

Store at +4C

#7239

PathScan® Cell Growth Multi-Target Sandwich ELISA Kit

Cell Signaling TECHNOLOGY®

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Species Cross Reactivity: H M

UniProt ID: #P27361, #P62753, #P28482, #P31749

Entrez-Gene Id: #5595, #6194, #5594, #207

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

Product Includes	Product #	Quantity	Color	Storage Temp
Cell Growth Multi-Target	66422	96 tests		+4C
TMB Substrate	7004	11 ml	Colorless	+4C
STOP Solution	7002	11 ml	Colorless	+4C
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-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

CST's PathScan® Cell Growth Multi-Target Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that combines the reagents necessary to detect endogenous levels of S6 ribosomal protein, phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phospho-Akt (Thr308) and phospho-p44/42 MAPK (Thr202/Tyr204). These molecules represent key signaling proteins in pathways controlling growth and differentiation. Sixteen assays are provided for each target protein. Specific assay formulations for the indicated target proteins can be found in the datasheets associated with the individual sandwich ELISA kits*. Briefly, a capture antibody** has been coated onto the microwells. After incubation with cell lysates, the target protein is captured by the coated antibody. Following extensive washing, a detection antibody** is added to detect the captured target protein. An HRP-linked secondary antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of absorbance for this developed color is proportional to the quantity of bound target protein. *See companion products. **Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity

CST's PathScan® Cell Growth Multi-Target Sandwich ELISA Kit #7239 detects endogenous levels of six proteins: S6 ribosomal protein, phospho-S6 ribosomal protein (Ser235/236), Akt1, phospho-Akt (Ser473), phosho-Akt (Thr308) and phospho-p44/42 MAPK (Thr202/Tyr204). Activation of these proteins can be observed over time in response to PDGF. As shown in Figure 1, stimulation of serum-starved NIH/3T3 cells with PDGF promotes phosphorylation of Akt1 at Thr308 and Ser473, S6 ribosomal protein at Ser235/236 and p44/42 MAPK at Thr202/Tyr204. The level of each target protein (phospho and nonphospho) remains unchanged throughout the 80 minute time course as demonstrated by Western analysis.
The relationship between the protein concentration of the lysate and the absorbance at 450 nm can be found in the datasheets associated with the individual PathScan® Sandwich ELISA Kits*. *See companion products.
This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background

Akt is a protooncogene with a critical regulatory role in diverse cellular processes including growth, survival and the cell cycle. Akt is also a major regulator of insulin signaling and glucose metabolism (1-4). Akt is activated by PI3 kinase signaling and activation loop phosphorylation at Thr308 by PDK1 and by phosphorylation within the carboxy terminus at Ser473 by the mTOR-rictor complex (TORC1) (5-7). Both p44 and p42 MAP kinases (Erk1 and Erk2) function in a protein kinase cascade that plays a critical role in the regulation of cell growth and differentiation (8-13). MAP kinases are activated by a wide variety of extracellular signals including growth and neurotrophic factors, cytokines, hormones and neurotransmitters. Activation of MAP kinases occurs through phosphorylation of threonine and tyrosine (202 and 204 of human MAP kinase or 183 and 185 of rat MAP kinase) at the sequence T*EY* by a single upstream MAP kinase kinase (MEK) (14,15).

To effectively promote growth and cell division in a sustained manner, growth factors and mitogens must upregulate translation (16,17). Growth factors and mitogens induce the activation of p70 S6 kinase, which in turn phosphorylates the S6 ribosomal protein. Phosphorylation of S6 ribosomal protein correlates with an increase in translation, particularly of mRNAs with an oligopyrimidine tract in their 5' untranslated regions (17). This group of mRNAs (5'TOP) encodes proteins involved in cell cycle progression and proteins that are part of the translational machinery, such as ribosomal proteins and elongation factors (17,18). The main *in vivo* S6 ribosomal protein phosphorylation sites, including Ser235, Ser236, Ser240 and Ser244, are located within a small 19 amino acid region in the S6 carboxy terminus (19,20).

Background References

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Trademarks and Patents

Limited Uses

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PathScan® Cell Growth Multi-Target Sandwich ELISA Kit

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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