Revision 1

Store at 4C and -20C

#7203

PathScan® Total S6 Ribosomal Protein Sandwich ELISA Antibody Pair

Cell Signaling TECHNOLOGY®

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

Species Cross Reactivity: H M

UniProt ID: #P62753

Entrez-Gene Id: #6194

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

Description

CST's PathScan® Total S6 Ribosomal Protein Sandwich ELISA Antibody Pair is offered as an alternative to our PathScan® Total S6 Ribosomal Protein Sandwich ELISA Kit #7225. Capture and Detection antibodies (100X stocks) and an HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are provided for performing 4 x 96 well ELISAs. The S6 Ribosomal Protein Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added, followed by S6 Ribosomal Protein Detection Antibody and HRP-conjugated secondary antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance at 450 nm is proportional to the quantity of Total S6 Ribosomal protein.
Antibodies in kit are custom formulations specific to kit.

Reagents Not Supplied

Phosphate Buffered Saline (PBS-20X) #9808 Phosphate Buffered Saline with Tween -20 (PBST-20X) #9809 Cell Lysis Buffer (10X) #9803 TMB Substrate #7004 STOP Solution #7002 Blocking Buffer-PBS+0.05% Tween-20, 1% BSA 96 Well Microplates** Microplate Reader ** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Background

One way that growth factors and mitogens effectively promote sustained cell growth and proliferation is by upregulating mRNA translation (1,2). Growth factors and mitogens induce the activation of p70 S6 kinase and the subsequent phosphorylation of S6 ribosomal protein. Phosphorylation of S6 ribosomal protein correlates with an increase in translation of mRNA transcripts that contain an oligopyrimidine tract in their 5' untranslated regions (2). These particular mRNA transcripts (5'TOP) encode proteins involved in cell cycle progression, as well as ribosomal proteins and elongation factors necessary for translation (2,3). Important S6 ribosomal protein phosphorylation sites include several residues (Ser235, Ser236, Ser240, and Ser244) located within a small, carboxy-terminal region of S6 protein (4,5).

Background References

1. Dufner, A. and Thomas, G. (1999) *Exp Cell Res* 253, 100-9.
2. Peterson, R.T. and Schreiber, S.L. (1998) *Curr Biol* 8, R248-50.
3. Jefferies, H.B. et al. (1997) *EMBO J* 16, 3693-704.
4. Ferrari, S. et al. (1991) *J Biol Chem* 266, 22770-5.
5. Flotow, H. and Thomas, G. (1992) *J Biol Chem* 267, 3074-8.

Trademarks and Patents

Limited Uses

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.

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ELISA Antibody Pair

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 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **Wash Buffer**: 1X PBS/0.05% Tween® 20, (20X PBST #9809).
3. **Blocking Buffer**: 1X PBS/0.05% Tween® 20, 1% BSA.
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.

5. **Bovine Serum Albumin (BSA)**: (#9998).
6. **TMB Substrate**: (#7004).
7. **STOP Solution**: (#7002)

   NOTE: Reagents should be made fresh daily.

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. Coating Procedure

1. Rinse microplate with 200 µl of dH_2O, discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in 1X PBS. For a single 96 well plate, add 100 µl of capture antibody stock to 9.9 ml 1X PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17–20 hr).
3. **After overnight coating, gently uncover plate and wash wells**:
   1. Discard plate contents into a receptacle.
   2. Wash four times with wash buffer, 200 µl each time per well. 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.
   3. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of blocking buffer/well, cover plate, and incubate at 37°C for 2 hr.
5. After blocking, wash plate (Section C, Step 3). Plate is ready to use.

D. Test Procedure

1. Lysates can be used undiluted or diluted in blocking buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hr.

2. Wash plate (Section C, Step 3).
3. Dilute detection antibody 1:100 in blocking buffer. For a single 96 well plate, add 100 µl of detection antibody Stock to 9.9 ml of blocking buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hr.
4. Wash plate (Section C, Step 3).
5. Secondary antibody, either streptavidin anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in blocking buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of blocking buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30 min.
6. Wash plate (Section C, Step 3).
7. Add 100 µl of TMB substrate per well. Cover and incubate at 37°C for 10 min.
8. Add 100 µl of STOP solution per well. Shake gently for a few seconds.
9. Read plate on a microplate reader at absorbance 450 nm.
    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 January 2008

revised Sepetember 2013