Revision 1

Store at 4C and -20C

#7993

PathScan® Phospho-Lck (Tyr505) Sandwich ELISA Antibody Pair

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:	UniProt ID:	Entrez-Gene Id:
H	#P06239	#3932

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

Description

Cell Signaling Technology's PathScan® Phospho-Lck (Tyr505) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-Lck (Tyr505) Sandwich ELISA Kit #7941. Capture and detection antibodies (100X stocks) and an HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The phospho-Lck (Tyr505) rabbit capture antibody is coated onto a 96 well microplate overnight in PBS. After blocking, cell lysates are added followed by a total Lck mouse detection antibody and anti-mouse IgG, HRP-linked antibody. HRP substrate (TMB) is then added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-Lck (Tyr505) protein.

Background

Lck belongs to the Src-like non-receptor tyrosine kinase family with the typical Src family kinase structure: a unique amino terminal domain (Src homology 4 domain, SH4) followed by an SH3 domain, an SH2 domain, a kinase domain (SH1), and a carboxy-terminal negative regulatory domain (1). Lck activity is controlled by the interactions of SH2 and SH3 domains as well as tyrosine phosphorylation status of the activation loop (2,3). Lck is recruited to the T cell receptor (TCR) complex upon stimulation and activates downstream tyrosine kinases to initiate T cell signaling (4). Lck is also found to be involved in the regulation of mitochondrial apoptosis pathways and may be responsible for some anticancer drug induced apoptosis (5,6).

Background References

1. Palacios, E.H. and Weiss, A. (2004) *Oncogene* 23, 7990-8000.
2. Mustelin, T. and Taskén, K. (2003) *Biochem J* 371, 15-27.
3. Gervais, F.G. et al. (1993) *Mol Cell Biol* 13, 7112-21.
4. Straus, D.B. and Weiss, A. (1992) *Cell* 70, 585-93.
5. Belka, C. et al. (2003) *Oncogene* 22, 176-85.
6. Gruber, C. et al. (2004) *Biochem Pharmacol* 67, 1859-72.

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PathScan® Phospho-Lck (Tyr505) Sandwich ELISA Antibody Pair

Cell Signaling TECHNOLOGY®

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**: PathScan® Sandwich ELISA Lysis Buffer (#7018) 1X: This buffer is ready to use as is. 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 to 1 ml ice-cold PathScan® Sandwich ELISA Lysis Buffer (1X) #7018 plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 2 min.
4. Collect cell lysate in a clean tube.
5. Centrifuge for 10 min (14,000 x g) at 4°C and transfer the supernatant to a new tube. Store supernatant 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. Resuspend the cell pellet and incubate the tube on ice for 2 min.
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.

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