Store at 4°C

FastScan™ Phospho-SLP-76 (Ser376) ELISA Kit

#30794

1 Kit
(96 assays)

New 12/18

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
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Entrez-Gene ID #3937
UniProt ID #Q13094

For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H, M

Description: The FastScan™ Phospho-SLP-76 (Ser376) ELISA Kit is a sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of SLP-76 when phosphorylated at Ser376. To perform the assay, sample is incubated with a capture antibody conjugated with a proprietary tag and a second detection antibody linked to HRP, forming a sandwich with phospho-SLP-76 (Ser376) in solution. This entire complex is immobilized to the plate via an anti-tag antibody. The wells are then washed to remove unbound material. TMB is then added. The magnitude of observed signal is proportional to the quantity of phospho-SLP-76 (Ser376).

*Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: The FastScan™ Phospho-SLP-76 (Ser376) ELISA Kit detects endogenous levels of SLP-76 when phosphorylated at Ser376 as 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: SH2 domain-containing leukocyte protein of 76 kDa (SLP-76) is a hematopoietic adapter protein that is important in multiple biochemical signaling pathways and necessary for T cell development and activation (1). ZAP-70 phosphorylates SLP-76 and LAT as a result of TCR ligation. SLP-76 has amino-terminal tyrosine residues followed by a proline rich domain and a carboxy-terminal SH2 domain. Phosphorylation of Tyr113 and Tyr128 result in recruitment of the GEF Vav and the adapter protein Nck (2). TCR ligation also leads to phosphorylation of Tyr145, which mediates an association between SLP-76 and Itk, which is accomplished in part via the proline rich domain of SLP-76 and the SH3 domain of ITK (3). Furthermore, the proline rich domain of SLP-76 binds to the SH3 domains of Grb2-like adapter Gads (3,4). In resting cells, SLP-76 is predominantly in the cytosol. Upon TCR ligation, SLP-76 translocates to the plasma membrane and promotes the assembly of a multi-protein signaling complex that includes Vav, Nck, Itk and PLCγ1 (1). The expression of SLP-76 is tightly regulated; the protein is detected at very early stages of thymocyte development, increases as thymocyte maturation progresses, and is reduced as cells mature to CD4+ CD8+ double-positive thymocytes (5).

Product Includes	Item #	Kit Quantity	Color
FastScan™ ELISA Microwell Strip Plate*	53257	96 tests	
SLP-76 Rabbit Capture mAb	57997	1 each	Green (Lyophilized)
Phospho-SLP-76 (Ser376) Mouse HRP-linked mAb	73160	1 each	Red (Lyophilized)
FastScan™ ELISA Capture Antibody Diluent	16076	3 ml	Green
FastScan™ ELISA HRP Antibody Diluent	28120	3 ml	
TMB Substrate	7004	11 ml	
STOP Solution	7002	11 ml	
Sealing Tape	54503	1 each	
ELISA Wash Buffer (20X)	9801	25 ml	
FastScan™ ELISA Cell Extraction Buffer (5X)	69905	10 ml	
FastScan™ ELISA Cell Extraction Enhancer Solution (50X)	25243	1 ml	
FastScan™ ELISA Kit #30794 Positive Control	77595	2 each	

* 12 8-well modules -Each module is designed to break apart for 8 tests.

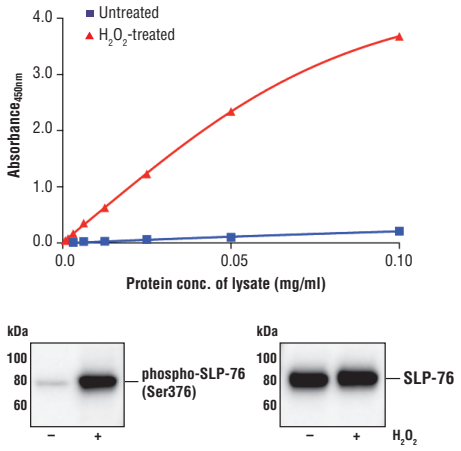

Figure 1. Treatment of Jurkat cells with H_2O_2 stimulates phosphorylation of SLP-76 at Ser376, but does not affect the level of total SLP-76 protein. The relationship between lysate protein concentration from untreated and H_2O_2-treated Jurkat cells and the absorbance at 450 nm using the FastScan™ Phospho-SLP-76 (Ser376) ELISA Kit #30794 is shown in the upper figure. The corresponding western blots using phospho-SLP-76 (Ser376) antibody (left panel) and SLP-76 antibody (right panel) are shown in the lower figure. After serum starvation, Jurkat cells were either left untreated or treated with 11 mM H_2O_2 for 3 minutes at 37°C and then lysed.

Background References:

(1) Clements, J.L. (2003) *Immunol Rev* 191, 211-9.
(2) Bubeck Wardenburg, J. et al. (1998) *Immunity* 9, 607-16.
(3) Bunnell, S.C. et al. (2000) *J Biol Chem* 275, 2219-30.
(4) Liu, S.K. et al. (1999) *Curr Biol* 9, 67-75.
(5) Clements, J.L. et al. (1998) *J Immunol* 161, 3880-9.

U.S. Patents 9,086,407, 9,261,500, and 9,476,874, foreign equivalents, and child patents deriving therefrom.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#30794

FastScan™ ELISA Protocol

A Solutions and Reagents

NOTE: Prepare solutions with deionized/purified water or equivalent. Prepare only as much reagent as needed on the day of the experiment.

1. **FastScan™ ELISA Microwell Strip Plate, 96 well (#53257):** 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. **1X ELISA Wash Buffer:** Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) to 1X with deionized water.
3. **1X Cell Extraction Buffer:** Prepare by diluting FastScan™ ELISA Cell Extraction Buffer (5X) #69905 and FastScan™ ELISA Cell Extraction Enhancer Solution (50X) #25243* to 1X with deionized water. This buffer can be stored at 4°C for short-term use (1-2 weeks). To make 10 mL 1X Cell Extraction Buffer, combine 7.8 mL deionized water, 2 mL FastScan™ ELISA Cell Extraction Buffer (5X), and 200 µL FastScan™ ELISA Cell Extraction Enhancer Solution (50X). Alternatively, Enhancer Solution may be added to the Cell Extraction Buffer after extraction of cells or tissue. When using the 1X Cell Extraction Buffer as a sample diluent for the assay, it is recommended to equilibrate it to room temperature prior to use.

   ***IMPORTANT:** The provided FastScan™ ELISA Cell Extraction Enhancer Solution (50X) may precipitate when stored at 4°C. To dissolve, warm briefly at 37°C and mix gently. The FastScan™ ELISA Cell Extraction Enhancer Solution (50X) can be stored at room temperature to avoid precipitation.

   NOTE: The 1X Cell Extraction Buffer contains phosphatase inhibitors. Protease inhibitors should be added to the 1X Cell Extraction Buffer immediately prior to lysing cells. Additional phosphatase inhibitors can also be added (e.g. Protease/Phosphatase Inhibitor Cocktail (100X) #5872, not supplied).
4. **FastScan™ ELISA Capture Antibody Diluent:** Green diluent for reconstitution of the Capture Antibody.
5. **FastScan™ ELISA HRP Antibody Diluent:** Diluent (amber bottle) for reconstitution of the HRP-linked Antibody. Protect from light.
6. **4X Capture Antibody:** Reconstitute lyophilized Capture Antibody (green colored cake) with 3 mL FastScan™ ELISA Capture Antibody Diluent (green diluent). Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. For best results, use immediately following antibody reconstitution. Unused reconstituted 4X Capture 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.
7. **4X HRP-linked Antibody:** Reconstitute lyophilized HRP-linked Antibody (red colored cake) with 3 mL FastScan™ ELISA HRP Antibody Diluent. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. For best results, use immediately following antibody reconstitution. Unused reconstituted 4X HRP-linked Antibody may be stored for up to 4 weeks at 4°C protected from light, although there may be some loss of signal compared to freshly reconstituted antibody.
8. **Antibody Cocktail:** Combine equal volumes of the reconstituted 4X Capture and 4X HRP-linked Antibodies immediately prior to assay and mix. To make 6 mL of the Antibody Cocktail (enough for 1x 96-well plate), combine 3 mL 4X Capture Antibody with 3 mL 4X HRP-linked Antibody.
9. **Positive Control:** Reconstitute 1 vial of lyophilized Positive Control by adding 250 µL deionized water. Mix thoroughly and gently, hold at room temperature for 1 minute and then follow the steps outlined below in the "Test Procedure" section. Positive Controls are recommended to be used immediately after reconstituting in deionized water, however remaining material may be stored at -80°C (there may be some loss of the positive control signal if freeze/thawed). Positive Controls are supplied as a control reagent, not as an absolute quantitation measure.
10. **TMB Substrate (#7004):** Bring to room temperature before use.
11. **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.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 mL ice-cold 1X Cell Extraction Buffer (recommended to supplement with protease inhibitors and additional phosphatase inhibitors as needed) 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 5 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 (~1200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/ml.
2. Wash once with ice-cold 1X PBS.
3. Cells harvested from 50 mL of growth media can be lysed in 2.0 mL of 1X Cell Extraction Buffer (recommended to supplement with protease inhibitors and additional phosphatase inhibitors as needed).
4. Sonicate lysates on ice.
5. Microcentrifuge for 5 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

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 Extraction Buffer to a 2X protein concentration in order to achieve a final 1X protein concentration upon addition of the antibody cocktail. 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 or Positive Control to the appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate with the supplied sealing tape 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:
   a. Discard plate contents into a receptacle.
   b. Wash 3 times with 1X ELISA Wash Buffer, 200 µL each time for every well. After each wash, aspirate or decant from wells. Invert the plate and blot it against clean paper towels to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   c. 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:
   a. **Visual Determination:** Read within 30 min after adding STOP Solution.
   b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.