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Polyclonal Antibody to Lck [pY192]

Alternate names: p56

Catalog No.: BP7119

Quantity: 0.1 ml

Host: Rabbit

Immunogen: The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human Lck that contains tyrosine 192 (based on Swiss Protein database, accession number P06239). The sequence is conserved in mouse.

Applications: The antibody has been used for Western blotting. For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. Positive control used: Full length untagged recombinant active human Lck protein. Other applications not tested. Optimal dilutions of this antibody are dependent on conditions and should be determined by the user.
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Specificity: Lck (p56lck), a member of the Src family of non-receptor tyrosine protein kinases, is expressed predominantly in T-cells. Lck function is critical both for T-cell development in the thymus and activation of mature T-cells in the periphery by antigen. The activity of Lck is regulated by phosphorylation of multiple sites, including the two conserved residues, tyrosines 394 (equivalent to 418 in Src) and 505 (equivalent to 529 in Src). Phosphorylation of tyrosine 192 by Syk promotes activation of Lck by preventing the binding of the negative regulatory site, tyrosine 505. Reacts with Human Lck. Other species/Src family kinases that have not been tested include: mouse Src (100% homologous with the immunogen), chicken Src (79% homologous); Lck (79% homologous), Hck (79% homologous) and Lyn (93% homologous).

Add. Information: BP7119/ME0905
Western Blotting Procedure
1. Lyse approximately 107 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use. Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein

concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100oC.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4oC.
12. Incubate the blocked blot with primary antibody at a concentration of 0.1-1.0 _g/mL in Tris buffered saline supplemented with 3% BSA and 0.1% Tween 20 for 2 hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab')2 anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab')2 anti-rabbit IgG horseradish peroxidase conjugate in conjunction with
your chemiluminescence reagents and instrumentation.
Cell Lysis Buffer
Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM Na4P2O7
2 mM Na3VO4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a
0.3 M stock in DMSO)
or 1 mM AEBSF (water
soluble version of PMSF)
60 _g/mL aprotinin
10 _g/mL leupeptin
1 _g/mL pepstatin
(alternatively, protease inhibitor
cocktail such as Sigma catalog
number P2714 may be used)
Transfer Buffer
Formulation:
2.4 gm Tris base
14.2 gm glycine

200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4oC prior to use.
Tris Buffered Saline
Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl
Blocking Buffer
Formulation:
100 mL Tris buffered saline
5 gm BSA
0.1 mL Tween 20
Peptide Competition Experiment
Phosphorylation Site Specific Antibodies (PSSAs) have been developed to enable the specific and sensitive detection of phosphorylation of particular amino acid residues in target proteins, while circumventing the need for protein purification, phosphopeptide mapping or handling radioactivity. The specificity of a PSSA in each experimental system can be confirmed through peptide competition. In this technique, aliquots of antibody are pre-incubated with peptide containing the sequence of the phosphopeptide immunogen used to raise the PSSA and the corresponding non-phosphopeptide. Following preincubation with the peptide, each antibody preparation is then used as a probe in antibody-based detection methods, such as Western blotting, immunocytochemistry, flow cytometry, or ELISA. With a PSSA specific for the phosphorylated target protein, pre-incubation with an excess of peptide containing the sequence of the phosphopeptide immunogen will block all antigen binding sites, while pre-incubation with the corresponding non-phosphopeptide will not affect the antibody.
There have been developed a line of control peptides specifically for use in peptide competition experiments with our PSSAs. These peptides, available as separate catalog items, are provided in pairs which contain the sequences of the phosphopeptide immunogen and the corresponding non-phosphopeptide.
In performing the Peptide Competition Experiment, it is important to note that the optimal dilutions of both antibody and peptide should be determined empirically for each specific application. The optimal dilution of antibody in these procedures is below saturating, as determined by previous experiments in your system. If an optimal antibody dilution has not been determined in your system, please refer to the Suggested Working Dilution on the antibody Product Analysis Sheet for guidance on an appropriate starting dilution. The optimal dilution of peptide used in these procedures will depend on the overall affinity or avidity of the antibody, as well as the quantity of the target antigen. A 50-150 fold molar excess of peptide to antibody is found to be effective for most peptide competition experiments.
In the example presented below, the PSSA is used as a dilution of 1:1000 and the peptides are used at a concentration of 333 nM. The total volume of the phosphopeptide and non-phosphopeptide-pre-incubated antibody preparations is 2 mL, sufficient for probing Western blot strips, as well as for use in other antibody-based detection methods. Under these conditions, the molar excess of peptide to antibody is d?ł50.

Storage: Store at -20oC. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at -20oC. For shipment or short-term storage (up to one week), 2-8oC is sufficient.

General Readings:
1. Yu XZ, Levin SD, Madrenas J, Anasetti C. Lck is required for activation-induced T cell death after TCR ligation with partial agonists. J Immunol. 2004 Feb 1;172(3):1437-43. PubMed PMID: 14734719.

2. Mustelin, T. and K. Tasken (2003) Positive and negative regulation of T-cell activation through kinases and phosphatases. Biochem. J. 371(Pt 1):15-27.
3. Goldmann WH. p56(lck) Controls phosphorylation of filamin (ABP-280) and regulates focal adhesion kinase (pp125(FAK)). Cell Biol Int. 2002;26(6):567-71. PubMed PMID: 12171035.
4. Couture C, Songyang Z, Jascur T, Williams S, Tailor P, Cantley LC, et al. Regulation of the Lck SH2 domain by tyrosine phosphorylation. J Biol Chem. 1996 Oct 4;271(40):24880-4. PubMed PMID: 8798764.
5. Xu H, Littman DR. The kinase-dependent function of Lck in T-cell activation requires an intact site for tyrosine autophosphorylation. Ann N Y Acad Sci. 1995 Sep 7;766:99-116. PubMed PMID: 7486706.

Protocols:

Prepare three identical test samples, such as identical PVDF or nitrocellulose strips to which the protein of interest has been transferred. The test samples should be blocked using a blocking buffer, such as Tris buffered saline supplemented with 0.1% Tween 20, and either 5% BSA or 5% non-fat dried milk.
Prepare 6.5 mL of working antibody stock solution (1:1000 in this example) by adding 6.5 µL of antibody stock solution to 6.5 mL of buffer containing blocking protein, such as TBS supplemented with 0.1% Tween 20, and either 3% BSA or 3% non-fat dried milk.
Apportion the unused PSSA into working aliquots and store at d?20d?°C for future use (the stock PSSA contains 50% glycerol and will not freeze at this temperature).
Allow the lyophilized control peptides to reach room temperature, ideally under desiccation.
Reconstitute each of the control peptides (supplied at 0.1 mg/vial) to a concentration of 66.7 □M with nanopure water. For a peptide with a molecular mass of 1500 (stated on the peptide Product Analysis Sheet), reconstitution with 1 mL water yields a solution with a concentration of 66.7 □M.
Apportion the unused reconstituted peptide solutions into working aliquots and store at -20°C for future use.
Label 3 test tubes as follows:

tube 1: water only no peptide control
tube 2: phosphopeptide
tube 3: non-phosphopeptide

Into each tube, pipette the following components

tube 1: 2 mL diluted PSSA solution plus 10 µL nanopure water
tube 2: 2 mL diluted PSSA solution plus 10 µL phosphopeptide
tube 3: 2 mL diluted PSSA solution plus 10 µL non-phosphopeptide

Incubate the three tubes for 30 minutes at room temperature with gentle rocking. During this incubation, the peptides have the chance to bind to the combining site of the antibody.
At the end of the incubation step, transfer the contents of each of the three tubes to clean reaction vessels containing one of the three identical test samples.

For Western blotting strips:
Incubate the strips with the pre-incubated antibody preparations for 1 hour at room temperature or overnight at 4°C.
Wash each strip four times, five minutes each, to remove unbound antibody.
Transfer each strip to a new solution containing a labeled secondary antibody [e.g., goat F(ab')2 anti-rabbit IgG alkaline phosphatase conjugate or goat F(ab')2 anti-rabbit IgG

horseradish peroxidase conjugate.
Remove unbound secondary antibody by thorough washing, and develop the signal using your chemiluminescent reagents and instrumentation.
The signal obtained with antibody incubated with the “Water Only, No Peptide Control” (Tube 1), represents the maximum signal in the assay. This signal should be eliminated by pre-incubation with the “Phosphopeptide” (Tube 2), while pre-incubation with the “Non-Phosphopeptide” (Tube 3) should not impact the signal. If the “Phosphopeptide” only partially eliminates the signal, repeat the procedure using twice the volume of water or peptide solutions listed in Step 8. If partial competition is seen following pre-incubation with the “Non-Phosphopeptide”, repeat the procedure using half the volumes of water or peptide solutions listed in Step 8.