Anti-Phospho-LYN (Y397)/LCK (Y394)/HCK (Y411)/BLK (Y389) Antibody [PSH09-43] - BSA and Azide free

HA751290

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 58 kDa
Clone number:	PSH09-43

Description: The Src family of protein tyrosine kinases, which includes Src, Lyn, Fyn, Yes, Lck, Blk, and Hck, are important in the regulation of growth and differentiation of eukaryotic cells. Src activity is regulated by tyrosine phosphorylation at two sites, but with opposing effects. While phosphorylation at Tyr416 in the activation loop of the kinase domain upregulates enzyme activity, phosphorylation at Tyr527 in the carboxy-terminal tail by Csk renders the enzyme less active.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Tyr394 of human LCK.

Positive control: COLO205 cell lysate, TT cell lysate, Ramos serum starved for 16 hours cell lysate, Ramos serum starved for 16 hours add 12μg/mL human IgM (diluted in serum free medium) for 2 minutes cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 50mM sodium orthovanadate for 5 minutes cell lysate, PC-12 cell lysate, PC-12 treated with 1mM sodium orthovanadate for 30 minutes cell lysate, Ramos cells serum starved for 16 hours add 12μg/mL human IgM (diluted in serum free medium) for 2 minutes.

Subcellular location: Cell membrane, Cytoplasm, cytosol.

Database links: SwissProt: P07948 Human | P06239 Human | P08631 Human | P51451 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

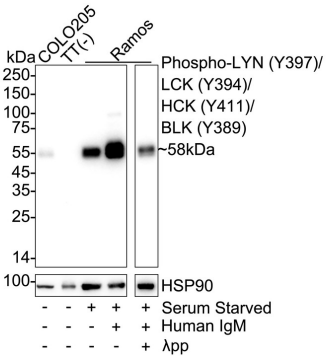

Fig1: Western blot analysis of Phospho-LYN (Y397)/LCK (Y394)/HCK (Y411)/BLK (Y389) on different lysates with Rabbit anti-Phospho-LYN (Y397)/LCK (Y394)/HCK (Y411)/BLK (Y389) antibody (HA751290) at 1/2,000 dilution.

Lane 1: COLO205 cell lysate
Lane 2: TT cell lysate (negative)
Lane 3: Ramos serum starved for 16 hours cell lysate
Lane 4: Ramos serum starved for 16 hours add 12μg/mL human IgM (diluted in serum free medium) for 2 minutes cell lysate
Lane 5: Ramos serum starved for 16 hours add 12μg/mL human IgM (diluted in serum free medium) for 2 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 μg/Lane.

Predicted band size: 58 kDa
Observed band size: 58 kDa

Exposure time: 23 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA751290) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

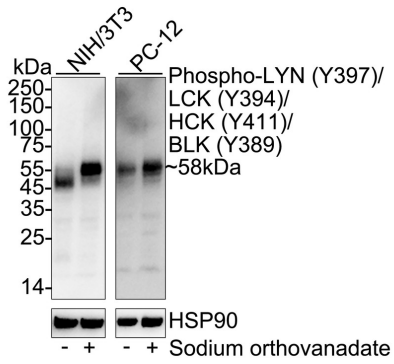

Fig2: Western blot analysis of Phospho-LYN (Y397)/LCK (Y394)/HCK (Y411)/BLK (Y389) on different lysates with Rabbit anti-Phospho-LYN (Y397)/LCK (Y394)/HCK (Y411)/BLK (Y389) antibody (HA751290) at 1/2,000 dilution.

Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
Lane 2: NIH/3T3 treated with 50mM sodium orthovanadate for 5 minutes cell lysate (20 µg/Lane)
Lane 3: PC-12 cell lysate (20 µg/Lane)
Lane 4: PC-12 treated with 1mM sodium orthovanadate for 30 minutes cell lysate (20 µg/Lane)

Predicted band size: 58 kDa
Observed band size: 58 kDa
Exposure time: Lane 1-2: 20 seconds; Lane 3-4: 1 minute; ECL: K1802;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA751290) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

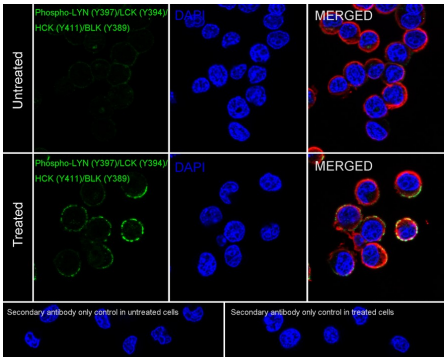

Fig3: Immunocytochemistry analysis of Ramos cells serum starved for 16 hours add 12µg/mL human IgM (diluted in serum free medium) for 2 minutes labeling Phospho-LYN (Y397)/LCK (Y394)/HCK (Y411)/BLK (Y389) with Rabbit anti-Phospho-LYN (Y397)/LCK (Y394)/HCK (Y411)/BLK (Y389) antibody (HA751290) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-LYN (Y397)/LCK (Y394)/HCK (Y411)/BLK (Y389) antibody (HA751290) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.