Anti-Phospho-Syk (Y525 + Y526) Antibody [PSH03-70] **HA722034**

Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human

Applications: WB, IF-Cell

Molecular Wt: Predicted band size: 72 kDa

Clone number: PSH03-70

Description: Tyrosine-protein kinase SYK, also known as spleen tyrosine kinase, is an enzyme which in

humans is encoded by the SYK gene. SYK, along with ZAP70, is a member of the Syk family of tyrosine kinases. These cytoplasmic non-receptor tyrosine kinases share a characteristic dual SH2 domain separated by a linker domain. However, activation of SYK relies less on phosphorylation by Src family kinases than ZAP70. SYK and ZAP70 share a common evolutionary origin and split from a common ancestor in the jawed vertebrates. While Syk and ZAP70 are primarily expressed in hematopoietic tissues, a variety of tissues express Syk. Within B and T cells, respectively, Syk and ZAP70 transmit signals from the B-cell receptor and T-cell receptor. Syk plays a similar role in transmitting signals from a variety of

cell surface receptors including CD74, Fc receptor, and integrins.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Tyr525 and 526 of

Human Syk.

Positive control: Ramos serum starved for 16 hours add 12µg/mL human IgM (diluted in serum free medium)

for 2 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: P43405 Human

Recommended Dilutions:

WB 1:1,000 **IF-Cell** 1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at $+4^{\circ}$ C after thawing. Aliquot store at -20° C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Images

HA722034 Competitor C kDa_1 2 kDa<u>,</u>1 250-150-250 150-100-72-55-100-72-55-Phospho-SYK (Y525/526) 45-35-45-35-25-25-14 1/1,000 1/1,000

Fig1: Western blot analysis of Phospho-Syk (Y525 + Y526) on different lysates with Rabbit anti-Phospho-Syk (Y525 + Y526) antibody (HA722034) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: Ramos cell lysate

Lane 2: Ramos serum starved for 16 hours add 12µg/mL human IgM (diluted in serum free medium) for 2 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 72 kDa Observed band size: 72 kDa

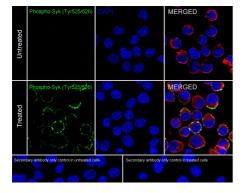
Exposure time: Lane 1-2 (left): 1 minute 2 seconds; Lane 1-2

(right): 2 minutes 18 seconds;

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 (HA722034) at 1/1,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at $4\,^{\circ}\mathrm{C}$ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of Ramos cells serum starved for 16 hours add $12\mu g/mL$ human IgM (diluted in serum free medium) for 2 minutes labeling Phospho-Syk (Y525 + Y526) with Rabbit anti-Phospho-Syk (Y525 + Y526) antibody (HA722034) at 1/1,000 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-Syk (Y525 + Y526) antibody (HA722034) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor **M 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 (M1305-2, red) was stained at 1/100 dilution overnight at $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor † 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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Background References

- Wang S et al. TREM2 drives microglia response to amyloid-β via SYK-dependent and -independent pathways. Cell. 2022 Oct
- 2. Ennerfelt H et al. SYK coordinates neuroprotective microglial responses in neurodegenerative disease. Cell. 2022

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