

**DESCRIPTION**

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human ZAP70 in Western blots.
<b>Source</b>	Polyclonal Sheep IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	<i>E. coli</i> -derived recombinant human ZAP70 Phe10-Cys102 Accession # P43403
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

**APPLICATIONS**

**Please Note:** Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Western Blot</b>	1 µg/mL	See Below
<b>Immunohistochemistry</b>	5-15 µg/mL	See Below
<b>Simple Western</b>	20 µg/mL	See Below

**DATA**

**Western Blot**

**Detection of Human ZAP70 by Western Blot.** Western blot shows lysates of Jurkat human acute T cell leukemia cell line, MOLT-4 human acute lymphoblastic leukemia cell line, and Ramos human Burkitt's lymphoma cell line. PVDF Membrane was probed with 1 µg/mL of Human ZAP70 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6375) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for ZAP70 at approximately 70 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

**Immunohistochemistry**

**ZAP70 in Human Lymphoma.** ZAP70 was detected in immersion fixed paraffin-embedded sections of human lymphoma using Human ZAP70 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6375) at 3 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to epithelial cell cytoplasm. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

**Simple Western**

**Detection of Human ZAP70 by Simple Western™.** Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line, MOLT-4 human acute lymphoblastic leukemia cell line, Ramos human Burkitt's lymphoma cell line, and Raji human Burkitt's lymphoma cell line, loaded at 0.2 mg/mL. A specific band was detected for ZAP70 at approximately 66 kDa (as indicated) using 20 µg/mL of Sheep Anti-Human ZAP70 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6375) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Sterile PBS to a final concentration of 0.2 mg/mL.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

#### BACKGROUND

ZAP70 (Zeta-associated protein 70 kDa; also Syk-related tyrosine kinase) is a 68-70 kDa member of the SYK subfamily, tyrosine kinase family of molecules. It is found in immature thymocytes, plus T cells and NK cells, and is essential for TCR-mediated IL-2 production, plus the development of CD8+ T cells. Human ZAP70 is 619 amino acids (aa) in length. It contains two SH2 domains (aa 10-102 and 162-254), a regulatory interdomain region (aa 255-337), and a C-terminal tyrosine kinase domain (aa 338-539). The two SH2 domains bind to phosphorylated tyrosines on the CD3  $\zeta$ -chain following TCR activation. This activates the ZAP70 kinase domain with the initiation of downstream signaling via SLP76 and LAT. There are four potential splice variants of ZAP70. One termed TZK is 37 kDa in size, and characterized by an alternative start site at Met308. This is expressed in very early thymocytes. Two others show an alternative start site 88 aa upstream of the standard site, with one of these variants also showing a deletion of aa 495-541. A fourth variant contains an eight aa substitution for aa 1-134. Over aa 10-102, human ZAP70 shares 98% aa identity with mouse ZAP70.