

DESCRIPTION

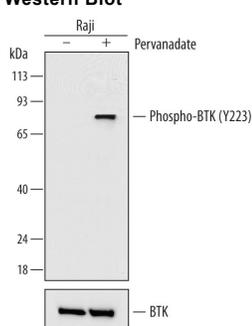
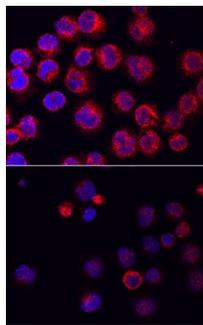
Species Reactivity	Human
Specificity	Detects human Phospho-BTK (Y223) in direct ELISAs and Western blots.
Source	Monoclonal Mouse IgG _{2B} Clone # 720101
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Phosphopeptide containing the human BTK Y223 site
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied 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.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below

DATA

<p>Western Blot</p>  <p>Detection of Human Phospho-BTK (Y223) by Western Blot. Western blot shows lysates of Raji human Burkitt's lymphoma cell line untreated (-) or treated (+) with 1 mM Pervanadate for 30 minutes. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human Phospho-BTK (Y223) Monoclonal Antibody (Catalog # MAB7205) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Phospho-BTK (Y223) at approximately 80 kDa (as indicated). For additional reference, duplicate samples were probed with 0.05 µg/mL of Mouse Anti-Human BTK Monoclonal Antibody (<i>lower panel</i>, Catalog # MAB5807) This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>Phospho-BTK (Y223) in Raji Human Cell Line. BTK phosphorylated at Y223 was detected in immersion fixed Raji human Burkitt's lymphoma cell line with (upper panel) and without (lower panel) pervanadate stimulation using Mouse Anti-Human Phospho-BTK (Y223) Monoclonal Antibody (Catalog # MAB7205) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.</p>
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PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
Shipping	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
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Bruton tyrosine kinase (BTK) is a 75 kDa cytoplasmic protein tyrosine kinase that is widely expressed in hematopoietic cells. BTK is required for B cell receptor signaling and B cell development. Defects in BTK result in X-linked agammaglobulinemia which is characterized by a severely decreased level of circulating antibodies. Like other Tec family kinases, BTK contains a Pleckstrin homology domain, a Tec homology domain, an SH3 domain, an SH2 domain, and a protein kinase domain. Association of the BTK SH2 domain with the B cell linker protein (BLNK) is required for the activation of PLCγ by BTK. The autophosphorylation of Tyr223 facilitates the interaction of BTK with activated Syk. The sequence used as a peptide immunogen is identical in human, mouse and rat BTK.