

Rabbit Anti-Phospho-BTK (Tyr223) antibody

SL3069R

Product Name:	Phospho-BTK (Tyr223)
Chinese Name:	磷酸化酪氨酸蛋白激酶BTK抗体
Alias:	BTK (phospho Y223); p-BTK (phospho Y223); Agammaglobulinaemia tyrosine kinase; AGMX 1; AGMX1; AT; ATK; B cell progenitor kinase; B-cell progenitor kinase; BPK; Bruton agammaglobulinemia tyrosine kinase; Bruton tyrosine kinase; Bruton's Tyrosine Kinase; Btk; BTK_HUMAN; IMD 1; IMD1; MGC126261; MGC126262; OTTHUMP00000063593; PSCTK 1; PSCTK1; Tyrosine protein kinase BTK; Tyrosine-protein kinase BTK; XLA.
Organism Species:	Rabbit
Clonality:	Polyclonal
React Species:	Human, Mouse, Rat, Pig, Cow, Horse, Rabbit,
Applications:	ELISA=1:500-1000IHC-P=1:400-800IHC-F=1:400-800Flow-Cyt=1ug/testICC=1:100-500IF=1:100-500 (Paraffin sections need antigen repair) not yet tested in other applications. optimal dilutions/concentrations should be determined by the end user.
Molecular weight:	76kDa
Cellular localization:	The nucleuscytoplasmicThe cell membrane
Form:	Lyophilized or Liquid
Concentration:	lmg/ml
immunogen:	KLH conjugated Synthesised phosphopeptide derived from human Btk around the phosphorylation site of Tyr223:AL(p-Y)DY
Lsotype:	IgG
Purification:	affinity purified by Protein A
Storage Buffer:	0.01M TBS(pH7.4) with 1% BSA, 0.03% Proclin300 and 50% Glycerol.
Storage:	Store at -20 °C for one year. Avoid repeated freeze/thaw cycles. The lyophilized antibody is stable at room temperature for at least one month and for greater than a year when kept at -20 °C. When reconstituted in sterile pH 7.4 0.01M PBS or diluent of antibody the antibody is stable for at least two weeks at 2-4 °C.
PubMed:	<u>PubMed</u>

Brutons tyrosine kinase (BTK) is a member of the BTK/Tec family of cytoplasmic tyrosine kinases. Like other BTK family members, it contains a pleckstrin homology (PH) domain, Src homology SH3 and SH2 domains. BTK plays an important role in B cell development. Activation of B cells by various ligands is accompanied by BTK membrane translocation mediated by its PH domain binding to phosphatidylinositol-3,4,5-trisphosphate. The membrane located BTK is active and associated with transient phosphorylation of two tyrosine residues, Tyr551 and Tyr223. Tyr551 in the activation loop is transphosphorylated by the Src family tyrosine kinase, leading to autophosphorylation at Tyr223 within the SH3 domain, which is necessary for full activation. The activation of BTK is negatively regulated by PKC beta through phosphorylation of BTK at Ser180, which results in reduced membrane recruitment, transphosphorylation and subsequent activation. The PKC/BTK inhibitory signal is likely to be a key determinant of the B cell receptor signaling threshold to maintain optimal BTK activity.

Function:

Non-receptor tyrosine kinase indispensable for B lymphocyte development, differentiation and signaling. Binding of antigen to the B-cell antigen receptor (BCR) triggers signaling that ultimately leads to B-cell activation. After BCR engagement and activation at the plasma membrane, phosphorylates PLCG2 at several sites, igniting the downstream signaling pathway through calcium mobilization, followed by activation of the protein kinase C (PKC) family members. PLCG2 phosphorylation is performed in close cooperation with the adapter protein B-cell linker protein BLNK. BTK acts as a platform to bring together a diverse array of signaling proteins and is implicated in cytokine receptor signaling pathways. Plays an important role in the function of immune cells of innate as well as adaptive immunity, as a component of the Toll-like receptors (TLR) pathway. The TLR pathway acts as a primary surveillance system for the detection of pathogens and are crucial to the activation of host defense. Especially, is a critical molecule in regulating TLR9 activation in splenic B-cells. Within the TLR pathway, induces tyrosine phosphorylation of TIRAP which leads to TIRAP degradation. BTK plays also a critical role in transcription regulation. Induces the activity of NF-kappa-B, which is involved in regulating the expression of hundreds of genes. BTK is involved on the signaling pathway linking TLR8 and TLR9 to NF-kappa-B. Transiently phosphorylates transcription factor GTF2I on tyrosine residues in response to BCR. GTF2I then translocates to the nucleus to bind regulatory enhancer elements to modulate gene expression. ARID3A and NFAT are other transcriptional target of BTK. BTK is required for the formation of functional ARID3A DNA-binding complexes. There is however no evidence that BTK itself binds directly to DNA. BTK has a dual role in the regulation of apoptosis.

Subunit:

Binds GTF2I through the PH domain. Interacts with SH3BP5 via the SH3 domain. Interacts with IBTK via its PH domain. Interacts with ARID3A, CAV1, FASLG, PIN1, TLR8 and TLR9.

Subcellular Location:

Product Detail:

Cytoplasm. Cell membrane; Peripheral membrane protein. Nucleus. Note=In steady state, BTK is predominantly cytosolic. Following B-cell receptor (BCR) engagement by antigen, translocates to the plasma membrane through its PH domain. Plasma membrane localization is a critical step in the activation of BTK. A fraction of BTK also shuttles between the nucleus and the cytoplasm, and nuclear export is mediated by the nuclear export receptor CRM1.

Tissue Specificity:

Predominantly expressed in B lymphocytes.

Post-translational modifications:

Following B-cell receptor (BCR) engagement, translocates to the plasma membrane where it gets phosphorylated at Tyr-551 by LYN and SYK. Phosphorylation at Tyr-551 is followed by autophosphorylation of Tyr-223 which may create a docking site for a SH2 containing protein. Phosphorylation at Ser-180 by PRKCB, leads in translocation of BTK back to the cytoplasmic fraction. Phosphorylation at Ser-21 and Ser-115 creates a binding site for PIN1 at these Ser-Pro motifs, and promotes it's recruitment.

DISEASE:

Defects in BTK are the cause of X-linked agammaglobulinemia (XLA) [MIM:300755]; also known as X-linked agammaglobulinemia type 1 (AGMX1) or immunodeficiency type 1 (IMD1). XLA is a humoral immunodeficiency disease which results in developmental defects in the maturation pathway of B-cells. Affected boys have normal levels of pre-B-cells in their bone marrow but virtually no circulating mature B-lymphocytes. This results in a lack of immunoglobulins of all classes and leads to recurrent bacterial infections like otitis, conjunctivitis, dermatitis, sinusitis in the first few years of life, or even some patients present overwhelming sepsis or meningitis, resulting in death in a few hours. Treatment in most cases is by infusion of intravenous immunoglobulin.

Defects in BTK may be the cause of X-linked hypogammaglobulinemia and isolated growth hormone deficiency (XLA-IGHD) [MIM:307200]; also known as agammaglobulinemia and isolated growth hormone deficiency or Fleisher syndrome or isolated growth hormone deficiency type 3 (IGHD3). In rare cases XLA is inherited together with isolated growth hormone deficiency (IGHD).

Similarity:

Belongs to the protein kinase superfamily. Tyr protein kinase family. TEC subfamily.

Contains 1 Btk-type zinc finger.

Contains 1 PH domain.

Contains 1 protein kinase domain.

Contains 1 SH2 domain.

Contains 1 SH3 domain.

SWISS:

O06187

Gene ID:

695

Database links:

Entrez Gene: 695Human

Entrez Gene: 12229Mouse

Omim: 300300Human

SwissProt: Q06187Human

SwissProt: P35991 Mouse

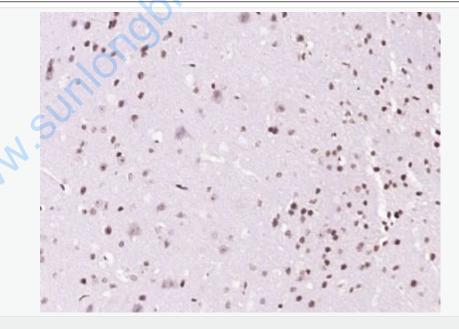
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Unigene: 4475Mouse

Important Note:

This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.

Picture:

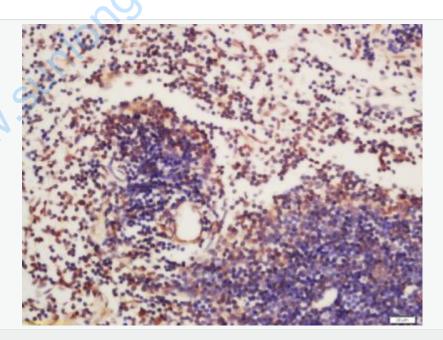


Paraformaldehyde-fixed, paraffin embedded (Mouse brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C

for 30min; Antibody incubation with (Phospho-BTK (Tyr223)) Polyclonal Antibody, Unconjugated (SL3069R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.

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Paraformaldehyde-fixed, paraffin embedded (Mouse colon); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-BTK (Tyr223)) Polyclonal Antibody, Unconjugated (SL3069R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.

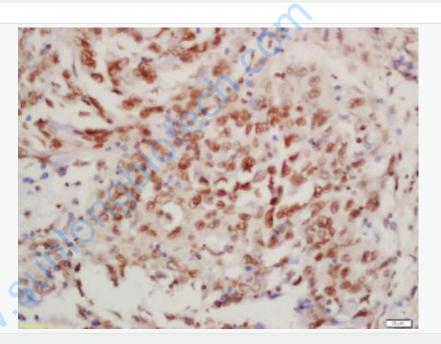


Tissue/cell: mouse spleen tissue; 4% Paraformaldehyde-fixed and paraffinembedded;

Antigen retrieval: citrate buffer (0.01M, pH 6.0), Boiling bathing for 15min; Block endogenous peroxidase by 3% Hydrogen peroxide for 30min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min;

Incubation: Anti-Phospho-BTK (Tyr223) Polyclonal Antibody,

Unconjugated(SL3069R) 1:200, overnight at 4°C, followed by conjugation to the secondary antibody(SP-0023) and DAB(C-0010) staining



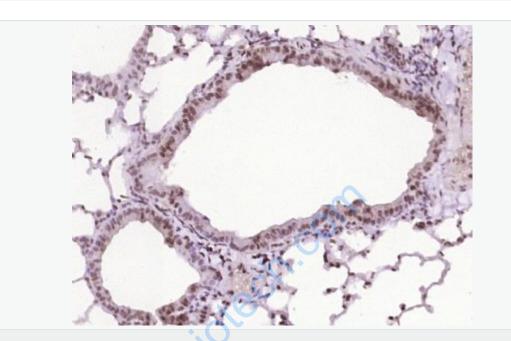
Tissue/cell: human lung carcinoma; 4% Paraformaldehyde-fixed and paraffinembedded;

Antigen retrieval: citrate buffer (0.01M, pH 6.0), Boiling bathing for 15min; Block endogenous peroxidase by 3% Hydrogen peroxide for 30min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min;

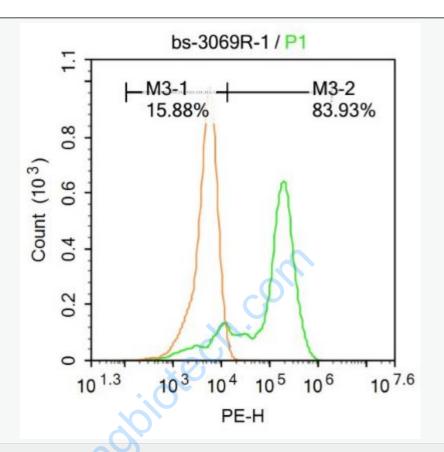
Incubation: Anti-Phospho-BTK (Tyr223) Polyclonal Antibody,

Unconjugated(SL3069R) 1:200, overnight at 4°C, followed by conjugation to the

secondary antibody(SP-0023) and DAB(C-0010) staining



Paraformaldehyde-fixed, paraffin embedded (Mouse lung); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-BTK (Tyr223)) Polyclonal Antibody, Unconjugated (SL3069R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.



U-937 cells were fixed with 4% PFA for 10min at room temperature, permeabilized with 90% ice-cold methanol for 20 min at room temperature, and incubated in 5% BSA blocking buffer for 30 min at room temperature. Cells were then stained with Phospho-BTK (Tyr223) Antibody(SL3069R)at 1:100 dilution in blocking buffer and incubated for 30 min at room temperature, washed twice with 2%BSA in PBS, followed by secondary antibody incubation for 40 min at room temperature. Acquisitions of 20,000 events were performed. Cells stained with primary antibody (green), and isotype control (orange).

