

Alexa Fluor® 647 anti-BTK Phospho (Tyr223) Antibody

Catalog# / Size	601707 / 25 tests 601708 / 100 tests
Clone	A16128B
Regulatory Status	RUO
Other Names	Bruton s tyrosine kinase, Bruton agammaglobulinemia tyrosine kinase, AGMX1, ATK, IMD1, PSCTK1, XLA, BPK
Isotype	Mouse IgG2a, κ
Description	Bruton's tyrosine kinase (BTK) is a Tec family kinase that plays a critical role in B cell development. Upon B cell receptor engagement, BTK translocates to the plasma membrane, where it is transphosphorylated by LYN and SYK kinases at Tyr 551. This initial phosphorylation event is followed by autophosphorylation at Tyr 223. Phosphorylated Tyr 223 is proposed to serve as a docking site for other proteins containing an SH2 domain. Downregulation of BTK activity is achieved through phosphorylation at Ser 180 by PKCβ, which results in reduced membrane recruitment and concomitant transphosphorylation. BTK-mediated B cell receptor signaling is required for B cell survival in the bone marrow and is a therapeutic target for certain B cell malignancies. Mutations in the <i>BTK</i> gene results in X-linked agammaglobulinemia (XLA), a primary immunodeficiency disease characterized by a failure in B cell maturation. Additionally, BTK plays a role in Toll-like receptor signaling in innate immune cells, and has been shown to play a direct role in NLRP3 inflammasome activation, thus contributing to ischaemic brain injury.

Product Details

Verified Reactivity	Human, Mouse
Antibody Type	Monoclonal
Host Species	Mouse
Immunogen	Human BTK peptide phosphorylated at Tyr 223, Complete Freund's adjuvant.
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)
Preparation	The antibody was purified by affinity chromatography and conjugated with Alexa Fluor® 647 under optimal conditions.
Concentration	Lot-specific (to obtain lot-specific concentration and expiration, please enter the lot number in our Certificate of Analysis online tool.)
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. Do not freeze.
Application	ICFC - Quality tested
Recommended Usage	Each lot of this antibody is quality control tested by intracellular flow cytometry using our True-Phos™ Perm Buffer in Cell Suspensions Protocol. For flow cytometric staining, the suggested use of this reagent is 5 µL per million cells in 100 µL staining volume or 5 µL per 100 µL of whole blood. It is recommended that the reagent be titrated for optimal performance for each application. * Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633 nm / 635 nm. Alexa Fluor® and Pacific Blue™ are trademarks of Life Technologies Corporation. View full statement regarding label licenses
Excitation Laser	Red Laser (633 nm)

Application Notes

A16128B was generated in the same immunization that produced clone A16128C (anti-BTK Phospho (Tyr223)/ITK Phospho (Tyr180)). Both clones bind BTK Phospho Tyr223 with similar affinity, but in comparison to clone A16128C, clone A16128B has weaker recognition of phosphorylated Tyrosine 180 of ITK by intracellular staining and flow cytometric analysis.

Antigen Details

Structure

BTK encodes a 659 amino acid protein with a predicted molecular weight of 76.2 kD. Contains a PH domain (a.a. 1-133) that binds phosphatidylinositol (3,4,5)-triphosphate (PIP3) and polyphosphates, an SH3 domain (a.a. 214-274), an SH2 domain (a.a. 281-377), and a protein kinase domain (402-655).

Distribution

Cytosol, plasma-membrane associated

Function

Links B cell receptor engagement with downstream signaling events

Interaction

ARID3A, BLNK, CAV1, GNAQ, GTF2I, PLGC2, PRKD1, SH3BP5

Ligand/Receptor

PIP3

Biology Area

Cell Biology, Signal Transduction

Molecular Family

Phospho-Proteins, Protein Kinases/Phosphatase

Antigen References

1. Krysiak K, *et al.* 2017. *Blood*. 129:473.
2. Burger JA, *et al.* 2016. *Nat Commun*. 7:11589.
3. Wang Q, *et al.* 2015. *Elife*. 4:e06074.
4. Ito M, *et al.* 2015. *Nat. Commun*. 6:7360.
5. Pillinger G, *et al.* 2015. *Sci. Rep*. 5:12949.
6. Cinar M, *et al.* 2013. *Leuk. Res*. 37:1271.
7. Doyle SL, *et al.* 2007 *J. Biol. Chem*. 282:36953.
8. Nore BF, *et al.* 2003. *Biochem. Biophys. Acta*. 1645:123.
9. Li Z, *et al.* 1997. *Proc. Natl. Acad. Sci*. 94:13820.

Gene ID

[695](#)

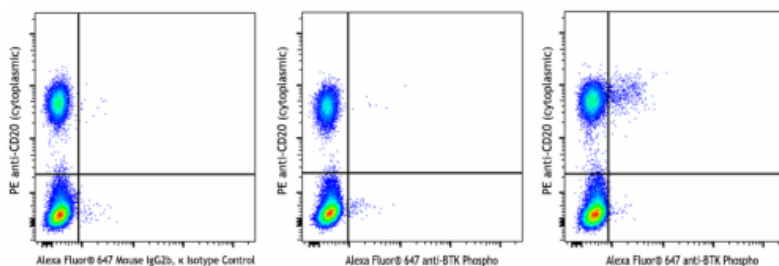
Related Protocols

- [Intracellular Staining With True-Phos™ Perm Buffer in Cell Suspensions Protocol](#)

Other Formats

PE anti-BTK Phospho (Tyr223), Alexa Fluor® 488 anti-BTK Phospho (Tyr223), Purified anti-BTK Phospho (Tyr 223), Alexa Fluor® 647 anti-BTK Phospho (Tyr223) Antibody

Product Data



PBMC untreated (middle panel) or treated with H₂O₂ (right panel) to induce phosphorylation of BTK were fixed and permeabilized using the True-Phos™ Perm Buffer Set (Cat. No. 425401) and intracellularly stained with Alexa Fluor® 647 anti-BTK Phospho (Tyr223) (clone A16128B) (middle and right panels) or Alexa Fluor® 647 Mouse IgG2a, κ Isotype Control (left panel, representative plot for untreated and H₂O₂-treated cells) (Cat. No. 400234). Cells were co-stained with PE anti-CD20 (cytoplasmic) (Cat. No. 340510).

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