abcam

ab247285

Anti-BTK (phospho Y551) antibody [EP267Y] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP267Y
Purification technique	Affinity purification Protein A
Specificity	*Stimulation may be required to allow detection of the phosphorylated protein. Please see images below for recommended treatment conditions and positive controls.* ab40770 also reacts with family proteins including BMX (phospho Y566), ITK (phospho Y512), TEC (phospho Y519) and TXK (phospho Y420).
Concentration	1.002 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

IP

Not recommended

Species	Human
Dilution info	-
Notes	-

Flow Cyt

Not recommended

Species	Human
Dilution info	-
Notes	-

Dot

Tested

Species	Human
Dilution info	1/1000
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

I-ELISA

Tested

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information BTK pY551

Function

Non-receptor tyrosine kinase indispensable for B lymphocyte development, differentiation and signaling (PubMed:19290921). Binding of antigen to the B-cell antigen receptor (BCR) triggers signaling that ultimately leads to B-cell activation (PubMed:19290921). 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 (PubMed:11606584). PLCG2 phosphorylation is performed in close cooperation with the adapter protein B-cell linker protein BLNK (PubMed:11606584). BTK acts as a platform to bring together a diverse array of signaling proteins and is implicated in cytokine receptor signaling pathways (PubMed:16517732, PubMed:17932028). 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 (PubMed:16517732). The TLR pathway acts as a primary surveillance system for the detection of pathogens and are crucial to the activation of host defense (PubMed:16517732). Especially, is a critical molecule in regulating TLR9 activation in splenic B-cells (PubMed:16517732, PubMed:17932028). Within the TLR pathway, induces tyrosine phosphorylation of TIRAP which leads to TIRAP degradation (PubMed:16415872). BTK also plays a critical role in transcription regulation (PubMed:19290921). Induces the activity of NF-kappa-B, which is involved in regulating the expression of hundreds of genes (PubMed:19290921). BTK is involved on the signaling pathway linking TLR8 and TLR9 to NF-kappa-B (PubMed:19290921). Acts as an activator of NLRP3 inflammasome assembly by mediating phosphorylation of NLRP3 (PubMed:34554188). Transiently phosphorylates transcription factor GTF2I on tyrosine residues in response to BCR (PubMed:9012831). GTF2I then translocates to the nucleus to bind regulatory enhancer elements to modulate gene expression (PubMed:9012831). ARID3A and NFAT are other transcriptional target of BTK (PubMed:16738337). BTK is required for the formation of functional ARID3A DNA-

binding complexes (PubMed:16738337). There is however no evidence that BTK itself binds directly to DNA (PubMed:16738337). BTK has a dual role in the regulation of apoptosis (PubMed:9751072). Plays a role in STING1-mediated induction of type I interferon (IFN) response by phosphorylating DDX41 (PubMed:25704810).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab247285 is the carrier-free version of ab40770.

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody

preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	BTK or Bruton's tyrosine kinase is an important enzyme in the body that functions mainly in signaling pathways within cells. It is known for its involvement in the development and activation of B cells and is expressed in cells of the hematopoietic system. The BTK protein has a molecular weight of about 76 kDa. Researchers can study BTK using tools like the BTK ELISA kit which helps measure its presence in biological samples. Furthermore there are specific anti-BTK antibodies which assist in the detection of BTK bands during analysis. BTK inhibitors like BTK C481S have been developed to regulate its activity.
Biological function summary	BTK plays an important role in the immune response by transmitting signals from the B cell receptor to the inside of the cell which promotes B cell maturation and survival. It operates as part of a larger signal transduction complex that includes other proteins and molecules. For accurate quantification of BTK expression a BTK sandwich ELISA kit may be utilized capturing and revealing the BTK protein's presence in sample preparations.
Pathways	BTK associates with both the B cell receptor signaling and the PI3K-Akt pathway. These pathways are critical for the proper functioning and proliferation of B cells. BTK interacts with proteins like PLCγ2 and BLNK in the signaling cascade highlighting its central role in transmitting extracellular signals to elicit appropriate cellular responses.
Associated diseases and disorders	BTK is closely linked to X-linked agammaglobulinemia (XLA) a genetic condition marked by an absence of mature B cells. This highlights the connection between BTK function and immune competence. Moreover aberrant BTK activity has been implicated in some types of B cell malignancies such as chronic lymphocytic leukemia (CLL). The BTK pathway's dysregulation in these disorders may involve interactions with proteins like SYK suggesting that targeting BTK could offer therapeutic benefits in managing such conditions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

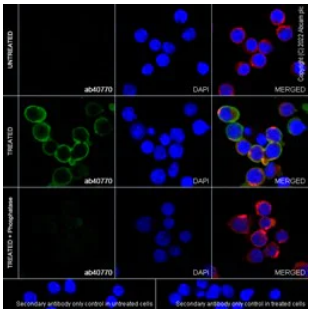
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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6 product images

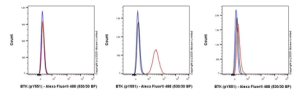


Immunocytochemistry/ Immunofluorescence - Anti-BTK (phospho Y551) antibody [EP267Y] - BSA and Azide free (ab247285)

BTK (phospho Y551) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-BTK (phospho Y551) antibody

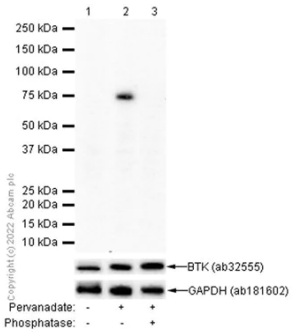
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Ramos (human Burkitt's lymphoma B lymphocyte) cells labelling BTK with primary antibody anti-BTK (phospho Y551) (ab40770) at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150081) secondary antibody at 1/1000 dilution (2.0 µg/mL). Confocal image showing increased cytoplasmic staining in Ramos cells treated with pervanadate (1 mM) for 30 min and then the signal decreased after Alkaline Phosphatase treatment 37☒ for 1 hour. Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) (ab195889) was used to counterstain tubulin at 1/200 dilution (2.5 µg/mL). The nuclear counter stain is DAPI (blue). The secondary antibody only control is : Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (2.0 µg/mL).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40770).



Flow Cytometry (Intracellular) - Anti-BTK (phospho Y551) antibody [EP267Y] - BSA and Azide free (ab247285)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40770).
Flow cytometric analysis of 4% paraformaldehyde fixed, 0.1% Tween-20 permeabilized Untreated control (Left) / Ramos cell treated with 1mM pervanadate for 30min (Middle) / Ramos cell treated with 1mM pervanadate for 30min, then incubated with Alkaline phosphatase overnight (Right) cells labelling BTK (phospho Y551) with ab40770 at 1/2000 dilution (0.1µg) followed by a Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at a 1/5000 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).



Western blot - Anti-BTK (phospho Y551) antibody [EP267Y] - BSA and Azide free (ab247285)

BTK (phospho Y551) Western blot staining using rabbit Anti-BTK (phospho Y551) antibody

Blocking buffer and concentration : 5% NFDM/TBST Diluting buffer and concentration : 5% NFDM/TBST This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40770).
Lanes 1 - 3:
Western blot - Anti-BTK (phospho Y551) antibody [EP267Y] - BSA and Azide free (ab247285) at 1/1000 dilution

Lanes 1 - 3:
Western blot - Anti-BTK (phospho Y551) antibody [EP267Y] (ab40770) at 1/1000 dilution

Lane 1:
Untreated K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate at 15 µg

Lane 2:
K-562 treated with 1mM pervanadate for 30 min whole cell lysate at 15 µg

Lane 3:
K-562 treated with 1mM pervanadate for 30 min whole cell lysate, then the membrane treated with Alkaline Phosphatase for 1 hour at 15 µg

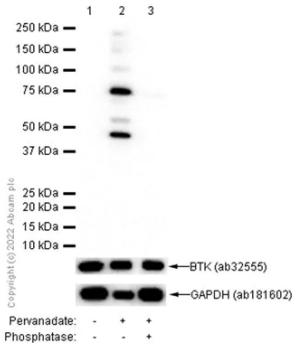
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 76 kDa

Observed band size: 75 kDa

Exposure time: 180s



Western blot - Anti-BTK (phospho Y551) antibody [EP267Y] - BSA and Azide free (ab247285)

BTK (phospho Y551) Western blot staining using rabbit Anti-BTK (phospho Y551) antibody

Blocking buffer and concentration : 5% NFDM/TBST Diluting buffer and concentration : 5% NFDM/TBST This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40770).
Lanes 1 - 3:
Western blot - Anti-BTK (phospho Y551) antibody [EP267Y] - BSA and Azide free (ab247285) at 1/1000 dilution

Lanes 1 - 3:
Western blot - Anti-BTK (phospho Y551) antibody [EP267Y] (ab40770) at 1/1000 dilution

Lane 1:
Untreated Ramos (Human Burkitt's lymphoma B lymphocyte) whole cell lysate at 15 µg

Lane 2:
Ramos treated with 1mM pervanadate for 30 min whole cell lysate at 15 µg

Lane 3:
Ramos treated with 1mM pervanadate for 30 min whole cell lysate, then the membrane treated with Alkaline Phosphatase for 1 hour at 15 µg

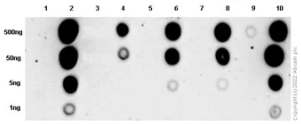
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 76 kDa

Observed band size: 75 kDa

Exposure time: 7s



Dot Blot - Anti-BTK (phospho Y551) antibody [EP267Y] - BSA and Azide free (ab247285)

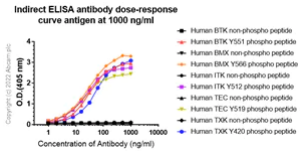
BTK (phospho Y551) Dot Blot staining using rabbit Anti-BTK (phospho Y551) antibody

Dot blot analysis of Human BTK non-phospho peptide (lane 1), Human BTK Y551 phospho peptide (lane 2), Human BMX non-phospho peptide (lane 3), Human BMX Y566 phospho peptide (lane 4), Human ITK non-phospho peptide (lane 5), Human ITK Y512 phospho peptide (lane 6), Human TEC non-phospho peptide (lane 7), Human TEC Y519 phospho peptide (lane 8), Human TXK non-phospho peptide (lane 9) and Human TXK Y420 phospho peptide (lane 10) with ab40770 at a dilution of 1/1000. ab97051 Peroxidase conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody at a dilution of 1/20,000.
Blocking buffer and concentration : 5% NFDM/TBST

Diluting buffer and concentration : 5% NFDM/TBST

Exposure time: 180 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40770).



Indirect ELISA - Anti-BTK (phospho Y551) antibody [EP267Y] - BSA and Azide free (ab247285)

ELISA using ab40770 at varying antibody concentrations and antigen concentration of 1000 ng/ml. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (1/2500) was used as the secondary antibody. Substrate solution was p-nitrophenyl phosphate (PNPP). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40770).

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.