abcam

ab227812

Anti-BTK antibody [EPR20445] - 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	EPR20445
Purification technique	Affinity purification Protein A
Concentration	1.065 - 1.077 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Rat, Mouse, Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Rat, Mouse
Dilution info	-
Notes	-

Target data

See full target information BTK

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

ab227812 is the carrier-free version of ab208937.

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

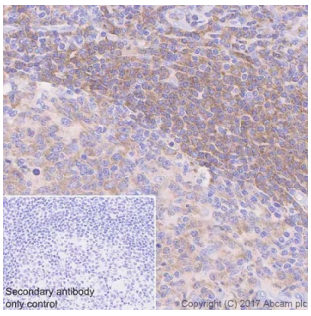
We do not recommend this combination. It is not covered by our product promise.

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.

12 product images



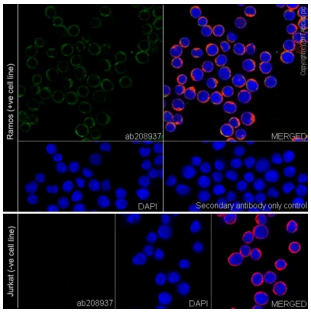
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

BTK Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-BTK antibody

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling BTK with ab208937 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Strong cytoplasmic staining in mantle zone and weaker cytoplasmic staining in the germinal center of human tonsil (PMID: 25433814). Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

BTK Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-BTK antibody

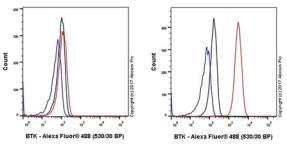
Immunofluorescent analysis of 100% methanol-fixed Ramos (human Burkitt's lymphoma cell line) and Jurkat (human T cell leukemia cell line from peripheral blood) cells labeling BTK with ab208937 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).
Confocal image showing cytoplasmic staining on Ramos cell line.

Negative control: Jurkat cell line (PMID: 24759210).

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).

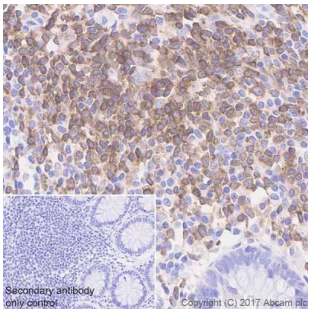


Flow Cytometry (Intracellular) - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

BTK Flow Cytometry (Intracellular) staining using rabbit Anti-BTK antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized Jurkat (human T cell leukemia cell line from peripheral blood) (left panel) and Ramos (human Burkitt's lymphoma cell line) (right panel) cell lines labeling BTK with ab208937 at 1/600 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.
Negative control: Jurkat cell line (PMID: 24759210).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).



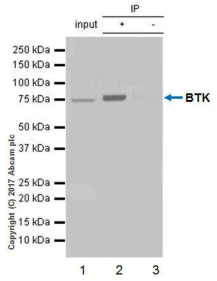
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

BTK Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-BTK antibody

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling BTK with ab208937 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Strong cytoplasmic staining on lymphoid nodule in human colon (PMID: 25433814). Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

BTK was immunoprecipitated from 0.35 mg of Ramos (human Burkitt's lymphoma cell line) whole cell lysate with ab208937 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab208937 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.
Lane 1: Ramos whole cell lysate 10 µg (Input).

Lane 2: ab208937 IP in Ramos whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab208937 in Ramos whole cell lysate.

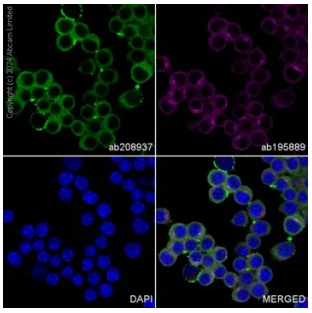
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).
All lanes:
Immunoprecipitation - Anti-BTK antibody [EPR20445] (ab208937)

Predicted band size: 76 kDa

Observed band size: 76 kDa



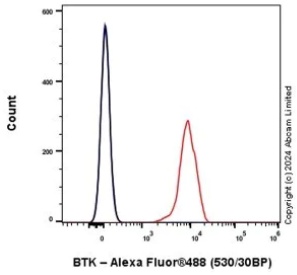
Immunocytochemistry/ Immunofluorescence - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

BTK Immunocytochemistry/ Immunofluorescence staining of RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells using rabbit Anti-BTK antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labeling BTK with ab208937 at 1/250 (8.6 ug/ml) dilution followed by ab150081, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed secondary antibody at 1/1000 (2 ug/ml) dilution (green). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used as a counterstain at 1/200 (2.5 ug/ml) dilution.

Confocal image showing cytoplasmic staining in RAW 264.7 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

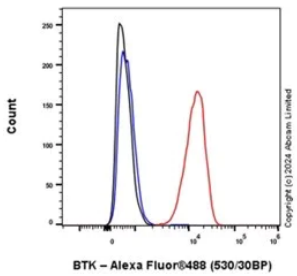


Flow Cytometry (Intracellular) - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cell lines labeling BTK with ab208937 at 1/50 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) was used as the secondary antibody at 1/5000 dilution.



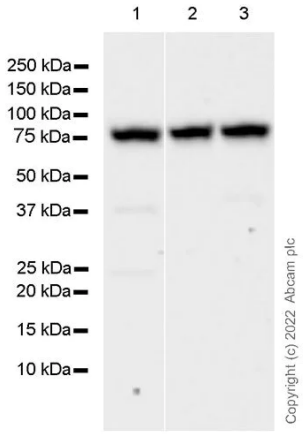
Flow Cytometry (Intracellular) - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized NR8383 (rat alveolar macrophage) cell lines labeling BTK with ab208937 at 1/50 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730)

(black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) was used as the secondary antibody at 1/5000 dilution.



Western blot - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

Blocking buffer and concentration: 5% NFDM/TBST **Diluting buffer and concentration:** 5% NFDM/TBST Lysates were freshly made and used for Western blotting immediately to minimize protein degradation. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).
All lanes:
Western blot - Anti-BTK antibody [EPR20445] (ab208937) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia cell) whole cell lysate at 20 µg

Lane 2:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 3:
NR8383 (rat lung macrophage (alveolar)) whole cell lysate at 20 µg

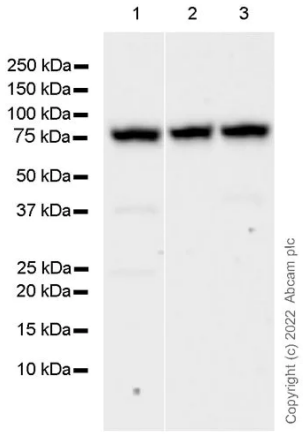
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 76 kDa

Observed band size: 76 kDa

Exposure time: 37s



Western blot - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

BTK Western blot staining using rabbit Anti-BTK antibody

Blocking buffer and concentration: 5% NFDM/TBST Diluting buffer and concentration: 5% NFDM/TBST Lysates were freshly made and used for Western blotting immediately to minimize protein degradation. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).
All lanes:
Western blot - Anti-BTK antibody [EPR20445] (ab208937) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia cell) whole cell lysate at 20 µg

Lane 2:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 3:
NR8383 (rat lung macrophage (alveolar)) whole cell lysate at 20 µg

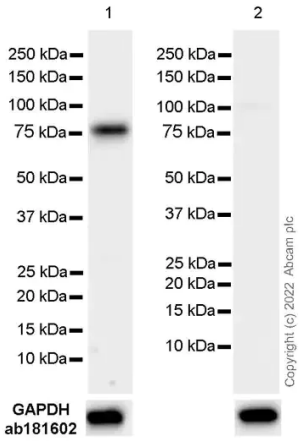
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 76 kDa

Observed band size: 76 kDa

Exposure time: 37s



Western blot - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

Blocking buffer and concentration: 5% NFDM/TBST **Diluting buffer and concentration:** 5% NFDM/TBST Lysates were freshly made and used for Western blotting immediately to minimize protein degradation. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).
All lanes:
Western blot - Anti-BTK antibody [EPR20445] (ab208937) at 1/1000 dilution

Lane 1:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 2:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

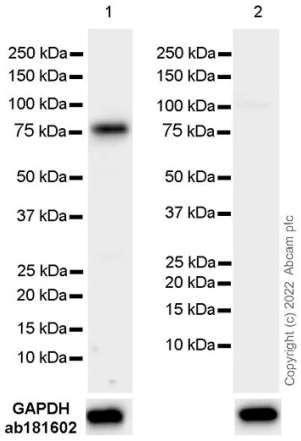
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 76 kDa

Observed band size: 76 kDa

Exposure time: 15s



Western blot - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

BTK Western blot staining using rabbit Anti-BTK antibody

Blocking buffer and concentration: 5% NFDM/TBST Diluting buffer and concentration: 5% NFDM/TBST Lysates were freshly made and used for Western blotting immediately to minimize protein degradation. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab208937).
All lanes:
Western blot - Anti-BTK antibody [EPR20445] (ab208937) at 1/1000 dilution

Lane 1:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 2:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Exposure time: 15s

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