abcam

Product datasheet

# Anti-BTK antibody [EPR20445] - BSA and Azide free ab227812

Recombinant RabMAb

8 Images

## Overview

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|---|---|
| **Product name** | Anti-BTK antibody [EPR20445] - BSA and Azide free |
| **Description** | Rabbit monoclonal [EPR20445] to BTK - BSA and Azide free |
| **Host species** | Rabbit |
| **Specificity** | This antibody does not react with mouse and rat species in Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) application. |
| **Tested applications** | **Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human |
| **Immunogen** | Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | IHC-P: Human tonsil and colon tissues. ICC/IF: Ramos cells. Flow Cyt (intra): Ramos cells. IP: Ramos whole cell lysate. WB: THP-1, RAW 264.7 and NR8383 whole cell lysates. |
| **General notes** | ab227812 is the carrier-free version of **ab208937**.<br><br>Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.<br><br>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.<br><br>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.<br><br>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.<br><br>This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**. |

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

## Properties

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| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C. Do Not Freeze. |
| **Storage buffer** | pH: 7.2<br>Constituent: PBS |
| **Carrier free** | Yes |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR20445 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab227812 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| **Flow Cyt (Intra)** | | Use at an assay dependent concentration. |
| **WB** | | Use at an assay dependent concentration. Detects a band of approximately 76 kDa (predicted molecular weight: 76 kDa). |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **ICC/IF** | | Use at an assay dependent concentration. |
| **IP** | | Use at an assay dependent concentration. |

## Target

**Function** Plays a crucial role in B-cell ontogeny. Transiently phosphorylates GTF2I on tyrosine residues in response to B-cell receptor cross-linking. Required for the formation of functional ARID3A DNA-binding complexes.

**Involvement in 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

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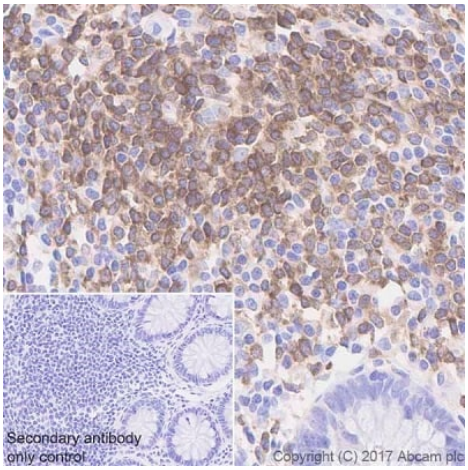
| | the first few years of life, or even some patients present overwhelming sepsis or meningitis, resulting in death in a few hours. Treatment in most cases is by infusion of intravenous immunoglobulin.<br>Defects in BTK may be the cause of X-linked hypogammaglobulinemia and isolated growth hormone deficiency (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). |
| **Sequence similarities** | Belongs to the protein kinase superfamily. Tyr protein kinase family. TEC subfamily.<br>Contains 1 Btk-type zinc finger.<br>Contains 1 PH domain.<br>Contains 1 protein kinase domain.<br>Contains 1 SH2 domain.<br>Contains 1 SH3 domain. |
| **Post-translational modifications** | Autophosphorylated on Tyr-223 and Tyr-551. Phosphorylation of Tyr-223 may create a docking site for a SH2 containing protein. |
| **Cellular localization** | Cytoplasm. Membrane. Nucleus. |

## Images



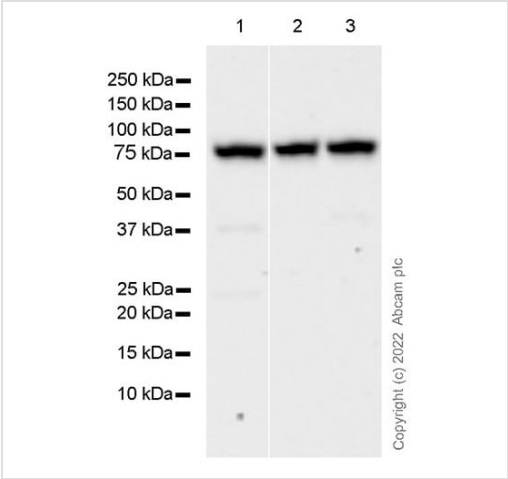

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

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.




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

**All lanes :** Anti-BTK antibody [EPR20445] (**ab208937**) at 1/1000 dilution

**Lane 1 :** THP-1 (human monocytic leukemia cell) whole cell lysate
**Lane 2 :** RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
**Lane 3 :** NR8383 (rat lung macrophage (alveolar)) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

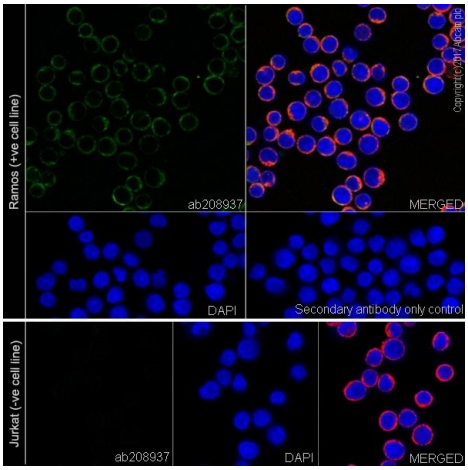
**Predicted band size:** 76 kDa
**Observed band size:** 76 kDa

**Exposure time:** 37 seconds

**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**).




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

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).

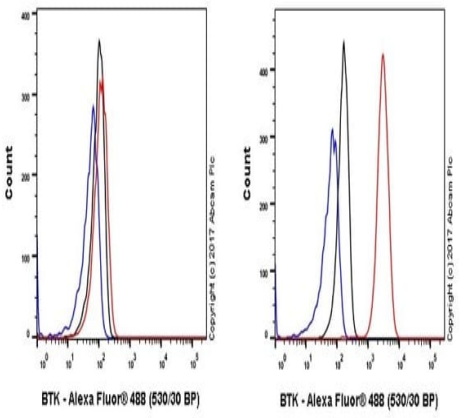
Confocal image showing cytoplasmic staining on Ramos cell line.

**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**).

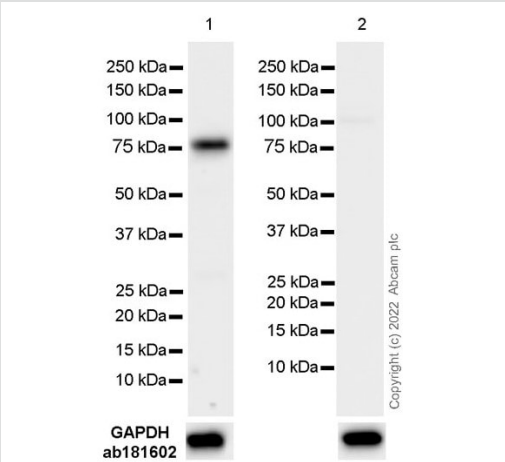



Flow Cytometry (Intracellular) - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

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**).




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

**All lanes :** 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
**Lane 2 :** NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

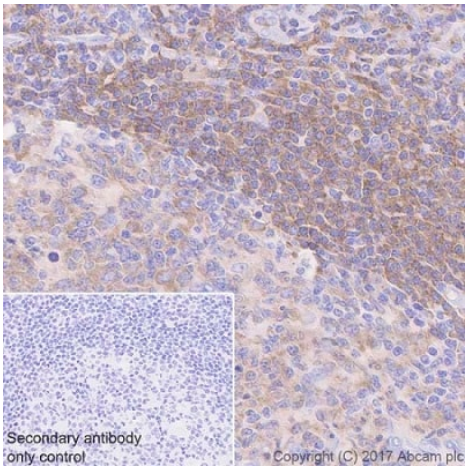
**Predicted band size:** 76 kDa
**Observed band size:** 76 kDa

**Exposure time:** 15 seconds

**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**).



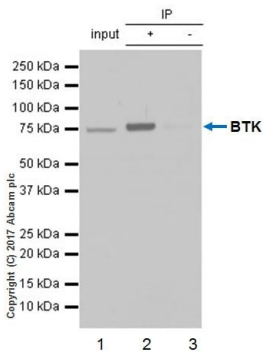

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BTK antibody [EPR20445] - BSA and Azide free (ab227812)

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.




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).

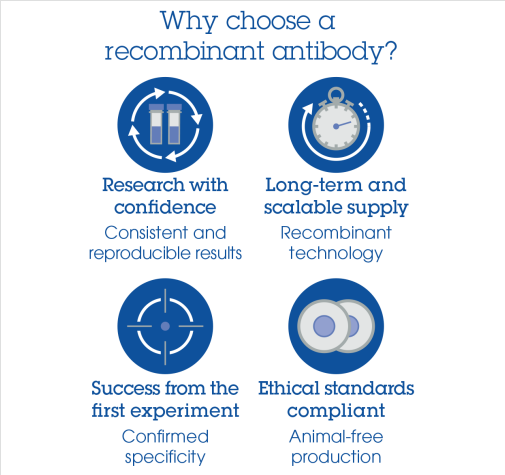
Lane 2: **ab208937** IP in Ramos whole cell lysate.

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**).




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

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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