# abcam

# Product datasheet

# Anti-BTK antibody [EPR20445] ab208937



重组 RabMAb

10 图像 6 References

概述

产品名称 Anti-BTK抗体[EPR20445]

描述 兔单克隆抗体[EPR20445] to BTK

宿主 Rabbit

特异性 This antibody does not react with mouse and rat species in Immunohistochemistry

(Formalin/PFA-fixed paraffin-embedded sections) application.

适用于: Flow Cyt (Intra), IHC-P, WB, ICC/IF, IP 经测试应用

与反应: Mouse, Rat, Human 种属反应性

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: Ramos, NAMALWA, THP-1, RAW 264.7 and NR8383 whole cell lysates; Human lymph node

lysate. IHC-P: Human tonsil and colon tissues. ICC/IF: Ramos cells. Flow Cyt (intra): Ramos cells.

IP: Ramos whole cell lysate.

常规说明 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 production

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**.

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯度 Protein A purified

克隆 单克隆

**克隆编号** EPR20445

同种型 IgG

#### 应用

# The Abpromise guarantee Abpromise™承诺保证使用ab208937于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/600.
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 76 kDa (predicted molecular weight: 76 kDa).
ICC/IF		1/100.
IP		1/30.

#### 靶标

## 功能

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.

## 疾病相关

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).

### 序列相似性

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.

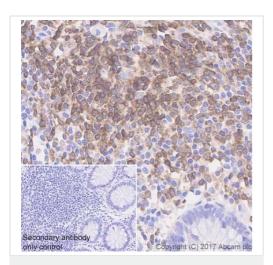
Contains 1 SH2 domain. Contains 1 SH3 domain.

## 翻译后修饰

Autophosphorylated on Tyr-223 and Tyr-551. Phosphorylation of Tyr-223 may create a docking

site for a SH2 containing protein.

## 图片

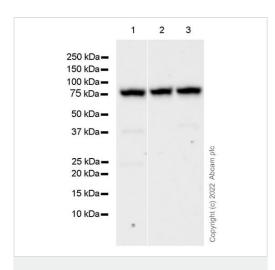


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BTK antibody
[EPR20445] (ab208937)

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 lgG H&L (HRP) Ready to use.

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



Western blot - Anti-BTK antibody [EPR20445] (ab208937)

**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 lgG H&L (HRP) (ab97051) at 1/100000 dilution

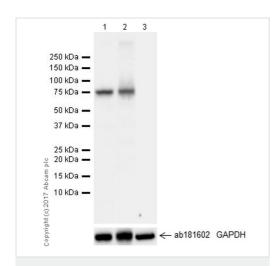
**Predicted band size:** 76 kDa **Observed band size:** 76 kDa

Exposure time: 37 seconds

**Blocking buffer and concentration:** 5% NFDM/TBST **Diluting buffer and concentration:** 5% NFDM/TBST

DAPI
Secondary antipody only control

Immunocytochemistry/ Immunofluorescence - Anti-BTK antibody [EPR20445] (ab208937)



Western blot - Anti-BTK antibody [EPR20445] (ab208937)

Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.

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 lgG H&L (Alexa Fluor<sup>®</sup> 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<sup>®</sup> 594) (**ab195889**) (red) at 1/200 dilution.

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

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

**Lane 1 :** Ramos (human Burkitt's lymphoma cell line) whole cell lysate

**Lane 2 :** NAMALWA (human Burkitt's lymphoma B lymphocyte) whole cell lysate

**Lane 3 :** Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

## Secondary

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

Developed using the ECL technique.

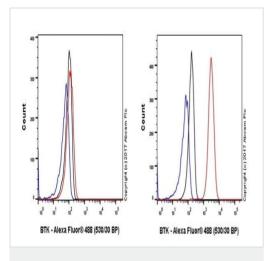
**Predicted band size:** 76 kDa **Observed band size:** 76 kDa

Exposure time: 32 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The Jurkat T cell line is negative for BTK (PMID 24759210). This image is performed on a BIO-RAD® ChemiDoc $^{\text{TM}}$  MP

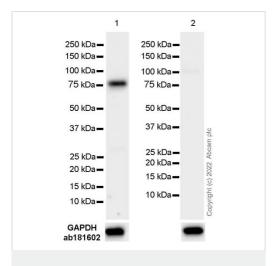
instrument.



Flow Cytometry (Intracellular) - Anti-BTK antibody [EPR20445] (ab208937)

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).



Western blot - Anti-BTK antibody [EPR20445] (ab208937)

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

**Lane 1 :** RAW 264.7 (mouse Abelson murine leukemia virusinduced 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 lgG 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.

250 kDa — 150 kDa — 100 kDa — 75 kDa — 37 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 10 kDa — 10 kDa —

Western blot - Anti-BTK antibody [EPR20445] (ab208937)

Anti-BTK antibody [EPR20445] (ab208937) at 1/1000 dilution + Human lymph node lysate at 10  $\mu g$ 

## Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

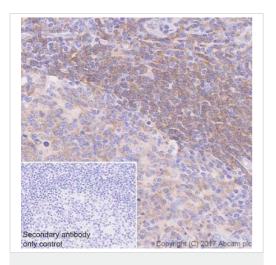
Developed using the ECL technique.

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

This image is performed on a BIO-RAD® ChemiDoc™ MP instrument.

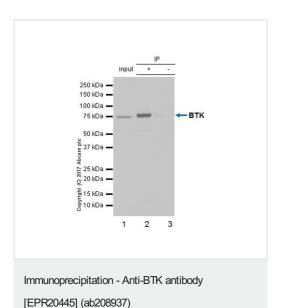


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BTK antibody
[EPR20445] (ab208937)

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 lgG H&L (HRP) Ready to use.

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



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.

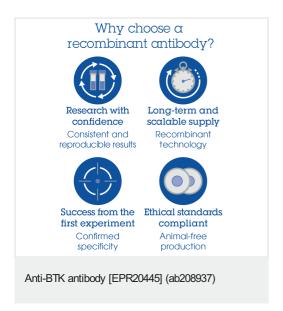
Lane 1: Ramos whole cell lysate 10 µg (Input).

Lane 2: ab208937 IP in Ramos whole cell lysate.

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

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

Exposure time: 3 seconds.



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