# abcam

# Product datasheet

# Anti-BTK (phospho Y223) antibody [EP420Y] ab68217

Recombinant RabMAb

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#### Overview

Product name Anti-BTK (phospho Y223) antibody [EP420Y]

**Description** Rabbit monoclonal [EP420Y] to BTK (phospho Y223)

Host species Rabbit

**Tested applications** Suitable for: WB, IP, Dot blot

Unsuitable for: ICC/IF or IHC-P

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as ab214589)

Positive control WB: K562 and Ramos cell lysates treated with pervanadate. IP: Ramos cell lysates treated with

pervanadate. Dot Blot: BTK (phospho Y223) phospho peptide.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

Improved sensitivity and specificityLong-term security of supplyAnimal-free production

For more information **see here**.

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

### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 50% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

**Clonality** Monoclonal

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Clone number EP420Y

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab68217 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
WB		1/2000 - 1/10000. Detects a band of approximately 76 kDa (predicted molecular weight: 76 kDa). Can be blocked with <u>BTK</u> <u>peptide (ab214589)</u> . For unpurified, use 1/10000 - 1/50000.
IP		1/20. For unpurified, use 1/40.
Dot blot		Use at an assay dependent concentration.

**Application notes** 

Is unsuitable for ICC/IF or IHC-P.

#### **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 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).

Sequence similarities

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.

Post-translational modifications

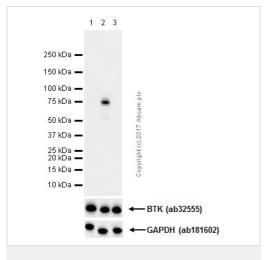
Autophosphorylated on Tyr-223 and Tyr-551. Phosphorylation of Tyr-223 may create a docking

site for a SH2 containing protein.

**Cellular localization** 

Cytoplasm. Membrane. Nucleus.

#### **Images**



Western blot - Anti-BTK (phospho Y223) antibody [EP420Y] (ab68217) **All lanes :** Anti-BTK (phospho Y223) antibody [EP420Y] (ab68217) at 1/1000 dilution

Lane 1 : Ramos (human Burkitt's lymphoma B lymphocyte) whole cell lysate

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

**Lane 3 :** Ramos treated with 1mM pervanadate for 30 min whole cell lysate. Then the membrane was incubated with alkaline phosphatase.

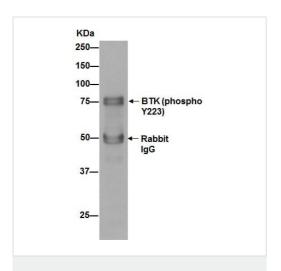
Lysates/proteins at 10 µg per lane.

# **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000 dilution

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

Blocking and dilution buffer: 5% NFDM/TBST.

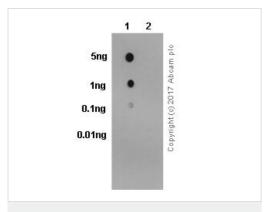


Immunoprecipitation - Anti-BTK (phospho Y223) antibody [EP420Y] (ab68217)

ab68217 (purified) at 1/50 immunoprecipitating BTK (phospho Y223) in Ramos cells (Lane 1) treated with 1 mM Pervanadate for 30 min. For western blotting, a HRP-conjugated anti-rabbit lgG (H+L) was used as the secondary antibody (1/1000).

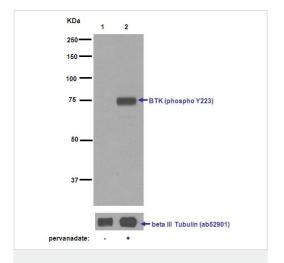
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Dot Blot - Anti-BTK (phospho Y223) antibody [EP420Y] (ab68217)

Dot blot analysis of BTK (phospho Y223) phospho peptide (Lane 1) and BTK phospho peptide (Lane 2) labeling BTK (phospho Y223) with ab68217 at a dilution of 1/1000. A Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) was used as the secondary antibody at a dilution of 1/20,000. Blocking buffer: 5% NFDM/TBST. Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-BTK (phospho Y223) antibody [EP420Y] (ab68217)

**All lanes :** Anti-BTK (phospho Y223) antibody [EP420Y] (ab68217) at 1/10000 dilution (purified)

Lane 1: Untreated K562 cell lysate

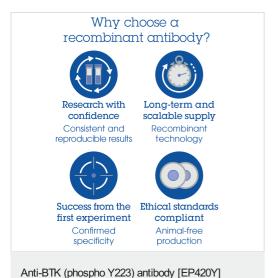
Lane 2: K562 cells treated with pervanadate

Lysates/proteins at 10 µg per lane.

#### **Secondary**

All lanes: HRP goat anti-rabbit (H+L) at 1/1000 dilution

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



(ab68217)

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