abcam

Product datasheet

Anti-Bcr antibody [EPR22062] ab222406

Recombinant RabMAb

8 Images

Overview

Product name	Anti-Bcr antibody [EPR22062]
Description	Rabbit monoclonal [EPR22062] to Bcr
Host species	Rabbit
Specificity	IHC is not recommended for human.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: K562, PC-12, NIH/3T3, MCF7 and HepG2 whole cell lysates; Human and rat brain tissue lysates; Human testis tissue lysate. IHC-P: Mouse hippocampus and rat cerebrum tissue. Flow Cyt (intra): K562 cells. IP: HeLa whole cell lysate.
General notes	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**.

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR22062
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab222406 in the following tested applications.

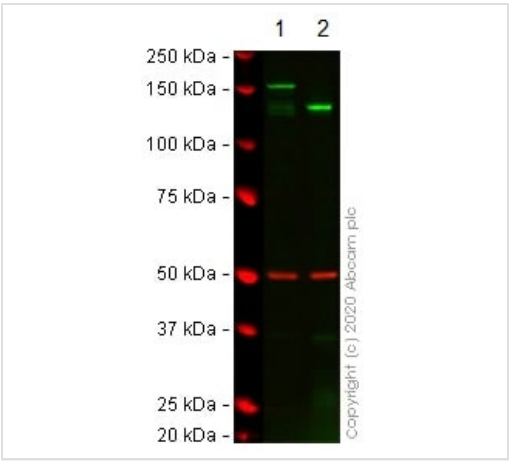
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 230, 210, 160, 130 kDa (predicted molecular weight: 143 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IP		1/30.

Target

Function	GTPase-activating protein for RAC1 and CDC42. Promotes the exchange of RAC or CDC42-bound GDP by GTP, thereby activating them. Displays serine/threonine kinase activity.
Involvement in disease	Note=A chromosomal aberration involving BCR is a cause of chronic myeloid leukemia. Translocation t(9;22)(q34;q11) with ABL1. The translocation produces a BCR-ABL found also in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).
Sequence similarities	Contains 1 C2 domain. Contains 1 DH (DBL-homology) domain. Contains 1 PH domain. Contains 1 Rho-GAP domain.
Domain	The region involved in binding to ABL1 SH2-domain is rich in serine residues and needs to be Ser/Thr phosphorylated prior to SH2 binding. This region is essential for the activation of the ABL1 tyrosine kinase and transforming potential of the chimeric BCR-ABL oncogene. The DH domain is involved in interaction with CCPG1.
Post-translational modifications	Autophosphorylated. Phosphorylated by FES/FPS on tyrosine residues, leading to down-regulation of the BCR kinase activity. Phosphorylation at Tyr-177 by HCK is important for interaction with GRB2.

Images



Western blot - Anti-Bcr antibody [EPR22062] (ab222406)

All lanes : Anti-Bcr antibody [EPR22062] (ab222406) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : BCR CRISPR/Cas9 edited HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

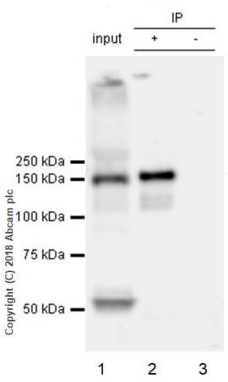
Performed under reducing conditions.

Predicted band size: 143 kDa
Observed band size: 150 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab222406 observed at 150 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab222406 was shown to react with BCR in wild-type HEK-293T cells in western blot. The bands observed in BCR CRISPR/Cas9 edited cell line **ab266583** (BCR CRISPR/Cas9 edited cell lysate **ab257858**) below 150kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and BCR CRISPR/Cas9 edited HEK-293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab222406 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4

176;® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



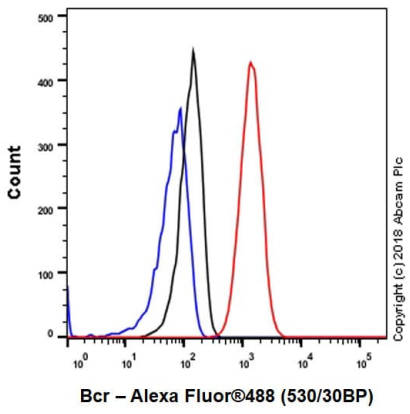
Immunoprecipitation - Anti-Bcr antibody [EPR22062] (ab222406)

Bcr was immunoprecipitated from 0.35 mg HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab222406 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab222406 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection antibody at 1/1000 dilution.

Lane 1: HeLa whole cell lysate 10µg (Input).
Lane 2: ab222406 IP in HeLa whole cell lysate (+)
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab222406 in HeLa whole cell lysate (-).

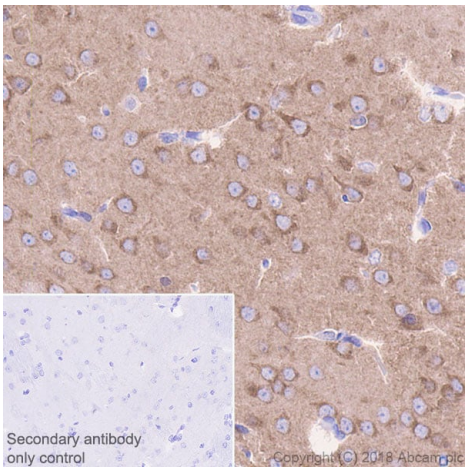
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 10 seconds.

The 160 kDa and 130 kDa bands are isoforms of BCR (PMID:2494632, PMID:3078961).



Flow Cytometry (Intracellular) - Anti-Bcr antibody [EPR22062] (ab222406)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized K562 (human chronic myelogenous leukemia cell line from bone marrow) cell line labeling Bcr with ab222406 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells incubated with secondary antibody only) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

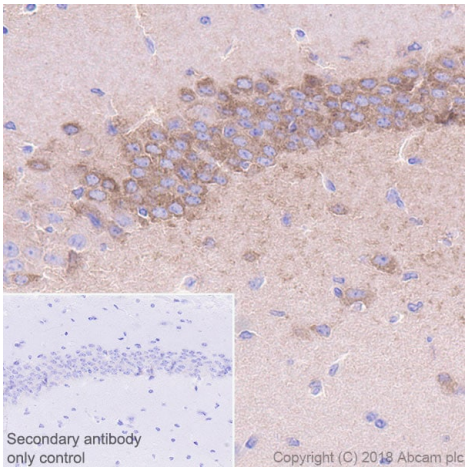


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcr antibody [EPR22062] (ab222406)

Immunohistochemical analysis of paraffin-embedded rat cerebrum tissue labeling Bcr with ab222406 at 1/500 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) Ready to use. Cytoplasmic staining in rat cerebrum (PMID: 25331951). Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) Ready to use.

Heat-mediated antigen retrieval using **ab208572** (Universal HIER antigen retrieval reagent).

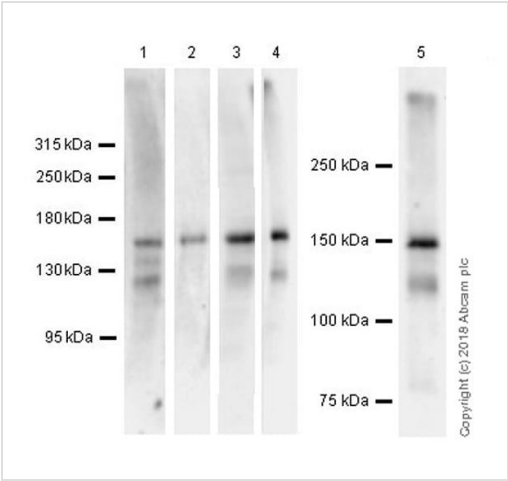


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Bcr antibody [EPR22062] (ab222406)

Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue labeling Bcr with ab222406 at 1/500 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) Ready to use. Cytoplasmic staining in mouse hippocampus (PMID: 25331951). Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) Ready to use.

Heat-mediated antigen retrieval using **ab208572** (Universal HIER antigen retrieval reagent).



Western blot - Anti-Bcr antibody [EPR22062] (ab222406)

All lanes : Anti-Bcr antibody [EPR22062] (ab222406) at 1/1000 dilution

Lane 1 : MCF7 (human breast adenocarcinoma cell line) whole lysate
Lane 2 : Human brain lysate
Lane 3 : HepG2 (human liver hepatocellular carcinoma cell line) whole lysate
Lane 4 : Rat brain lysate
Lane 5 : Human testis lysate

Lysates/proteins at 20 µg per lane.

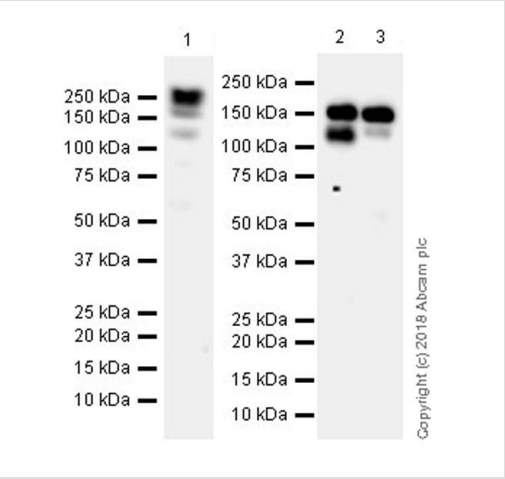
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 143 kDa
Observed band size: 130,160 kDa

Exposure time : Lanes 1 and 2: 3 minutes; Lanes 3 and 4: 59 seconds; Lane 3: 81 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

The 160 kDa and 130 kDa bands are isoforms of BCR (PMID: 2494632, PMID: 3078961).



Western blot - Anti-Bcr antibody [EPR22062] (ab222406)

All lanes : Anti-Bcr antibody [EPR22062] (ab222406) at 1/1000 dilution

Lane 1 : K562 (human chronic myelogenous leukemia cell line from bone marrow) whole lysate
Lane 2 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole lysate
Lane 3 : NIH/3T3 (mouse embyro fibroblast cell line) whole lysate

Lysates/proteins at 20 µg per lane.

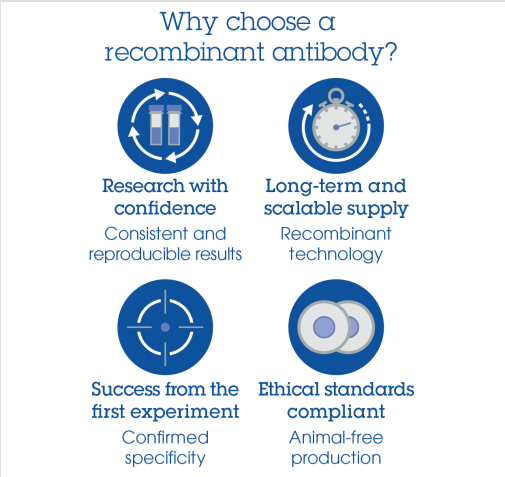
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 143 kDa
Observed band size: 130,160,210,230 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The 230/210 kDa band is the BCR-ABL fusion protein in K562 cells (PMID:17684099, PMID:16585609, PMID:12476301). The 160 kDa and 130 kDa bands are isoforms of BCR (PMID:2494632, PMID:3078961).



Anti-Bcr antibody [EPR22062] (ab222406)

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