abcam

Product datasheet

Anti-PTP1B antibody [EPR22474] - BSA and Azide free ab245984

KO VALIDATED Recombinant RabMAb

3 References 11 Images

Overview

Product name	Anti-PTP1B antibody [EPR22474] - BSA and Azide free
Description	Rabbit monoclonal [EPR22474] to PTP1B - BSA and Azide free
Host species	Rabbit
Specificity	IHC is recommended for human only.
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	WB: HeLa, Jurkat and MCF7 cell lysates. IHC-P: Human breast cancer and colon cancer tissue. ICC/IF: HeLa, HAP1 and HCT 116 cells. Flow Cyt (intra): HeLa and HCT 116 cells. IP: PTP1B IP in HCT 116 whole cell lysate.
General notes	ab245984 is the carrier-free version of **ab244207**. 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. 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. 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. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22474
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab245984 in the following tested applications.

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

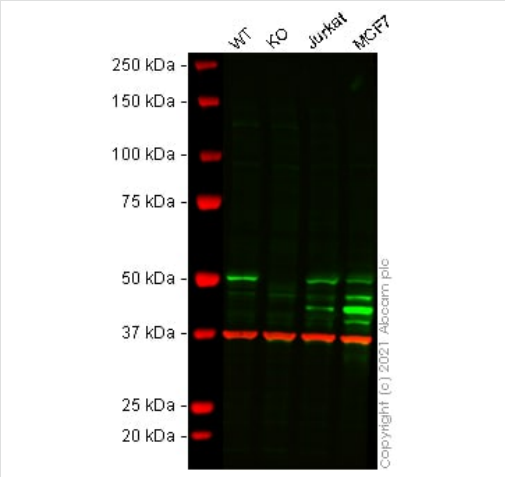
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 50 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. IHC is recommended for human only.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Target

Function	May play an important role in CKII- and p60c-src-induced signal transduction cascades.
Sequence similarities	Belongs to the protein-tyrosine phosphatase family. Non-receptor class 1 subfamily. Contains 1 tyrosine-protein phosphatase domain.
Post-translational modifications	Oxidized on Cys-215; the Cys-SOH formed in response to redox signaling reacts with the alpha-amido of the following residue to form a 4-amino-3-isothiazolidinone serine cross-link, triggering a conformational change that inhibits substrate binding and activity. The active site can be restored by reduction.

Cellular localization Endoplasmic reticulum membrane.

Images



Western blot - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

All lanes : Anti-PTP1B antibody [EPR22474] (**ab244207**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PTPN1 knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

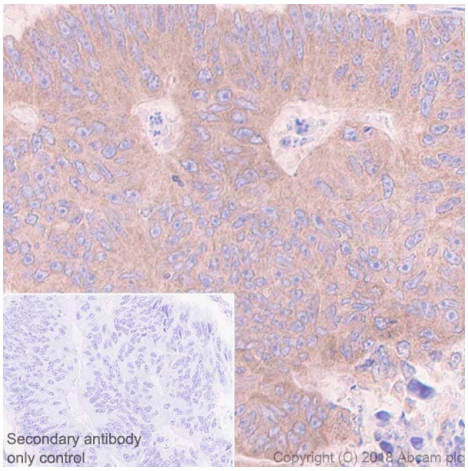
Performed under reducing conditions.

Predicted band size: 50 kDa
Observed band size: 51 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab244207**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab244207** observed at 51 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

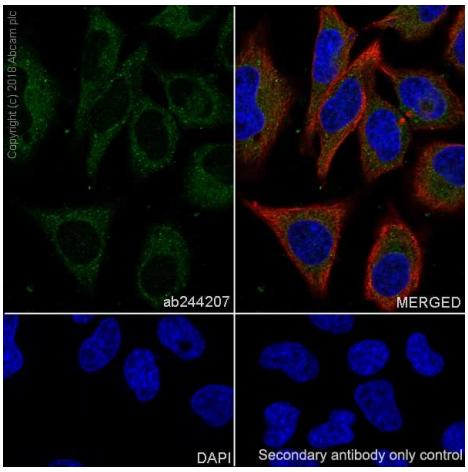
ab244207 was shown to react with PTP1B in wild-type HeLa cells in Western blot with loss of signal observed in PTPN1 knockout cell line **ab265014** (PTPN1 knockout cell lysate 257617). Wild-type HeLa and PTPN1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5 % milk in TBS-T (0.1 % Tween®) before incubation with **ab244207** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling PTP1B with **ab244207** at 1/1000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining in human colon cancer (PMID:27752061) is observed. Counterstained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).
Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

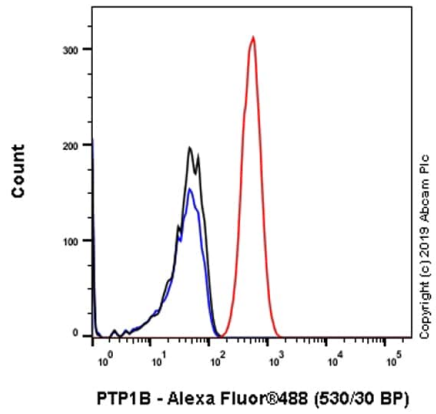
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab244207**).



Immunocytochemistry/ Immunofluorescence - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labeling PTP1B with **ab244207** at 1/50 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 in HeLa cells. The nuclear counterstain is DAPI (blue).
Counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (red).
The negative control is the secondary antibody only.

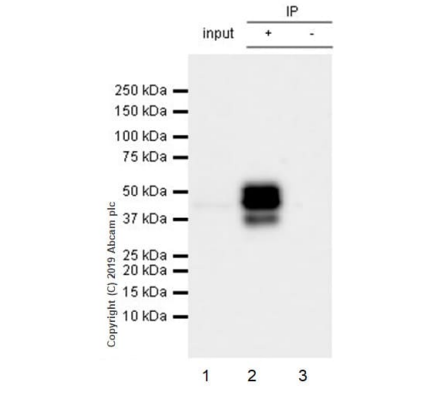
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab244207**).



Flow Cytometry (Intracellular) - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cell line labeling PTP1B with **ab244207** at 1/500 (red) compared with a Rabbit monoclonal IgG (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**), at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab244207**).



Immunoprecipitation - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

PTP1B was immunoprecipitated from 0.35 mg HCT 116 (human colorectal carcinoma epithelial cell) whole cell lysate with **ab244207** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab244207** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution.

Lane 1: HCT 116 whole cell lysate 10 µg (Input).

Lane 2: **ab244207** IP in HCT 116 whole cell lysate.

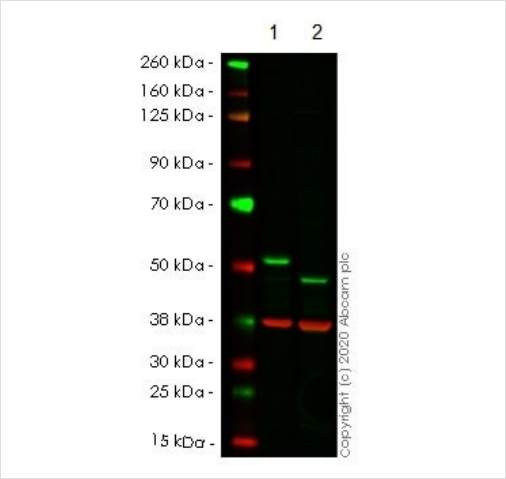
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab244207** in HCT 116 whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 30 seconds.

The observed MW is consistent with what described in the literatures. (PMID: 18253097; PMID: 11895943; PMID: 19797268).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab244207**).



Western blot - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

All lanes : Anti-PTP1B antibody [EPR22474] (**ab244207**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PTPN1 CRISPR/Cas9 edited HeLa cell lysate

Lysates/proteins at 20 µg per lane.

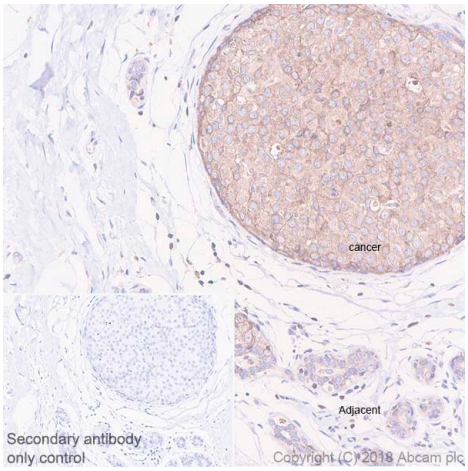
Performed under reducing conditions.

Predicted band size: 50 kDa
Observed band size: 50 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab244207**).

Lanes 1- 2: Merged signal (red and green). Green - **ab244207** observed at 50 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

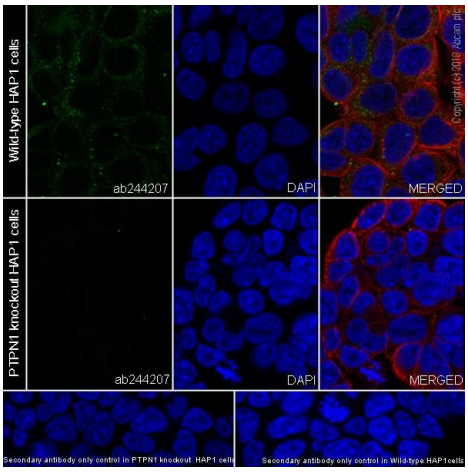
ab244207 was shown to react with PTP1B in wild-type HeLa cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab265014** (CRISPR/Cas9 edited cell lysate **ab257617**) lane below 50kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and PTPN1 CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab244207** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling PTP1B with **ab244207** at 1/1000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Higher cytoplasmic expression in human breast cancer than that of adjacent normal tissues (PMID: 27465552) is observed. Counterstained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).
Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

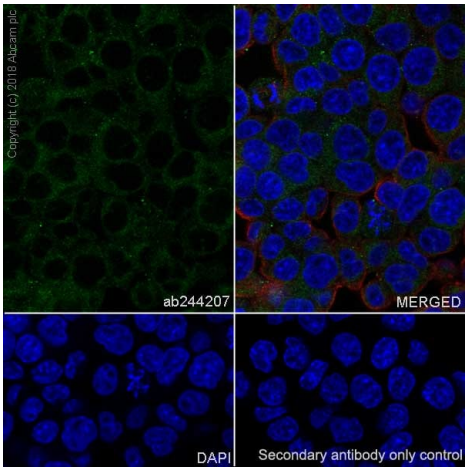
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab244207**).



Immunocytochemistry/ Immunofluorescence - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Wild type and PTP1B-knockout HAP1 (Human chronic myelogenous leukemia near-haploid cell line) cells labeling PTP1B with **ab244207** at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing no staining in PTP1B-knockout HAP1 cells. The nuclear counterstain is DAPI (blue).
Counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (red).
The negative control is the secondary antibody only.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab244207**).



Immunocytochemistry/ Immunofluorescence - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT 116 (human colorectal carcinoma epithelial cell) cells labeling PTP1B with **ab244207** at 1/50 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 in HCT 116 cells. The nuclear counterstain is DAPI (blue).
Counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (red).
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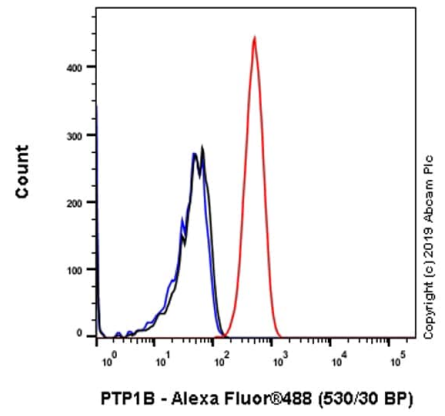
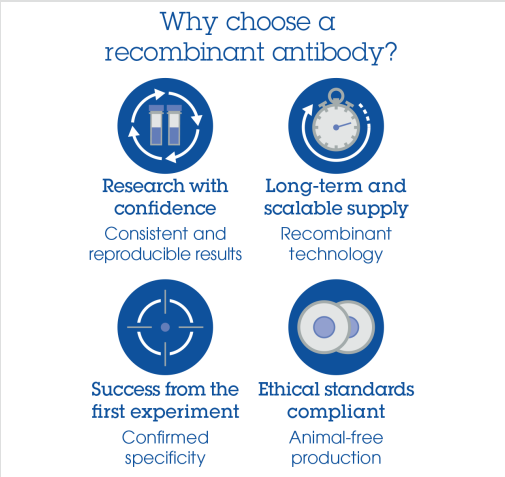


Flow Cytometry (Intracellular) - Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HCT 116 (human colorectal carcinoma epithelial cell) cell line labeling PTP1B with **ab244207** at 1/50 (red) compared with a Rabbit monoclonal IgG (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**), at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab244207**).



Anti-PTP1B antibody [EPR22474] - BSA and Azide free (ab245984)

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