abcam

Product datasheet

Anti-PTP1B antibody [EPR22474] ab244207



Recombinant RabMAb

7 References 14 Images

Overview

Product name Anti-PTP1B antibody [EPR22474]

Rabbit monoclonal [EPR22474] to PTP1B **Description**

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: HAP1, HeLa, HepG2, A549, MCF7, SW480, Jurkat, RAW 264.7, PC-12, NIH/3T3 and HCT

> 116 whole cell lysates. IHC-P: Human breast cancer and colon cancer tissue. ICC/IF: HeLa, wild type HAP1 and HCT 116 cells. Flow Cyt (intra): HeLa and HCT 116 cells. IP: PTP1B IP in HCT

116 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

ClonalityMonoclonalClone numberEPR22474

Isotype IgG

Applications

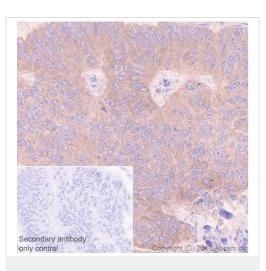
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab244207 in the following tested applications.

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

| Application | Abreviews | Notes |
|------------------|-----------|---|
| Flow Cyt (Intra) | | 1/50. |
| WB | | 1/1000. Predicted molecular weight: 50 kDa. |
| IHC-P | | 1/1000. 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 | | 1/50. |
| IP | | 1/30. |

| 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. | |
| | | |

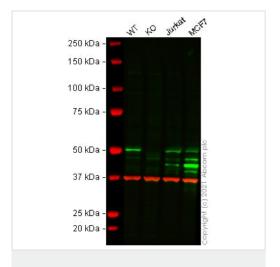
Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PTP1B antibody
[EPR22474] (ab244207)

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) secondary antibody. 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 <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-PTP1B antibody [EPR22474] (ab244207)

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.

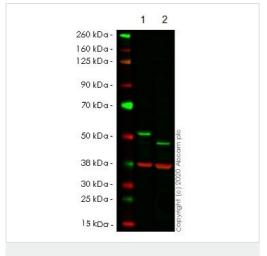
Performed under reducing conditions.

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

Lanes 1 - 4: Merged signal (red and green). Green - ab244207 observed at 51 kDa. Red - loading control <u>ab8245</u> (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 ab257617). 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 lgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-PTP1B antibody [EPR22474] (ab244207)

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

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 lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse

lgG H&L (IRDye[®]680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

ab244207

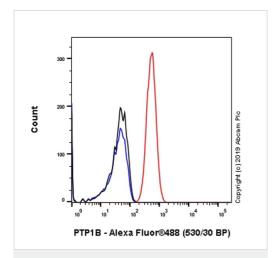
MERGED

DAPI

Secondary antibody only control

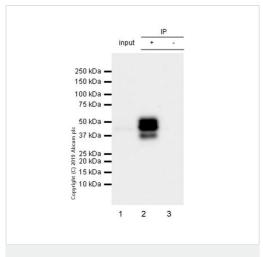
Immunocytochemistry/ Immunofluorescence - Anti-PTP1B antibody [EPR22474] (ab244207)

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 is observed. 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.

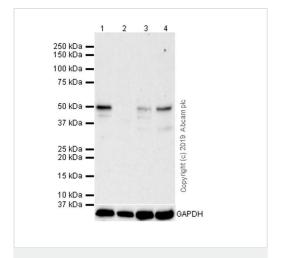


Flow Cytometry (Intracellular) - Anti-PTP1B antibody [EPR22474] (ab244207)

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 lgG (ab172730) isotype control (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit lgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-PTP1B antibody [EPR22474] (ab244207)



Western blot - Anti-PTP1B antibody [EPR22474] (ab244207)

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 lgG (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).

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

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: PTP1B knockout HAP1 whole cell lysate

Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 4 : HCT 116 (human colorectal carcinoma epithelial cell) 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: 50 kDa

ab244207 was shown to specifically react with PTP1B in wild-type HAP1 cells as signal was lost in PTP1B knockout cells. Wild-type and PTP1B knockout samples were subjected to SDS-PAGE. ab244207 and ab181602 (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-

RAD[®] ChemiDoc™ MP instrument using the ECL technique.

The expression profile observed is consistent with what has been described in the literature (PMID: 18253097; PMID: 11895943; PMID: 19797268). The bands below 50 kDa may represent truncated forms and cleaved fragments.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

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

Lane 1 : HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate

Lane 2: A549 (human lung carcinoma epithelial cell) whole cell lysate

Lane 3 : MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 4: SW480 (human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

1 2 3 4

250 kDa —

150 kDa —

100 kDa —

75 kDa —

37 kDa —

25 kDa —

20 kDa —

15 kDa —

10 kDa —

10 kDa —

Western blot - Anti-PTP1B antibody [EPR22474] (ab244207)

Secondary

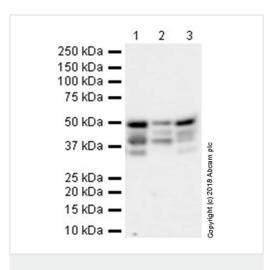
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 50 kDa

The expression profile observed is consistent with what has been described in the literature (PMID: 18253097; PMID: 11895943; PMID: 19797268). The bands below 50 kDa may represent truncated forms and cleaved fragments.

Blocking/Dilution buffer: NFDM/TBST.

Exposure time: 37 seconds.



Western blot - Anti-PTP1B antibody [EPR22474] (ab244207)

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

Lane 1 : RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) whole cell lysate

Lane 2: PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

Lane 3: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

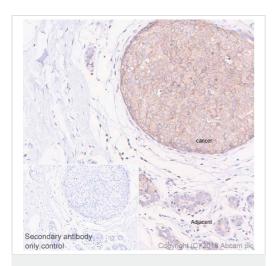
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 50 kDa

The expression profile observed is consistent with what has been described in the literature (PMID: 18253097; PMID: 11895943; PMID: 19797268). The bands below 50 kDa may represent truncated forms and cleaved fragments.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 48 seconds.



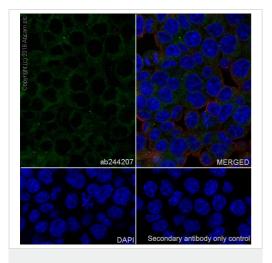
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PTP1B antibody
[EPR22474] (ab244207)

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 lgG 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).

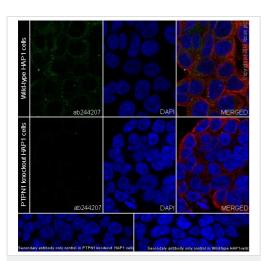
Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-PTP1B antibody [EPR22474] (ab244207)

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 a 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).

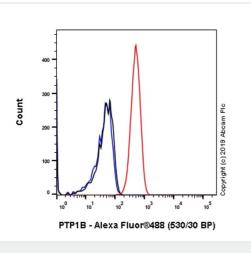
Counterstained with <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) at a 1/200 dilution (red). The negative control is the secondary antibody only.



Immunocytochemistry/ Immunofluorescence - Anti-PTP1B antibody [EPR22474] (ab244207)

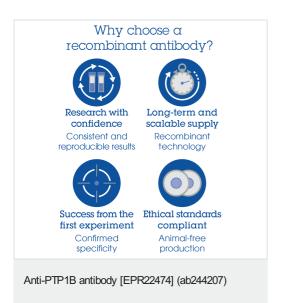
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Wild type and PTP1B-knockout HAP1 (Human chronic myelogenous leukemia near-haploid cell line) cells labeling PTP1B with ab244207 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) 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 <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor $^{\mbox{\it B}}$ 594) at a 1/200 dilution (red). The negative control is the secondary antibody only.



Flow Cytometry (Intracellular) - Anti-PTP1B antibody [EPR22474] (ab244207)

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) isotype control (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.



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