abcam

Product datasheet

Anti-PAI1 antibody [EPR17272-21] ab182973

Recombinant RabMAb

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Overview

Product name Anti-PAI1 antibody [EPR17272-21]

Description Rabbit monoclonal [EPR17272-21] to PAI1

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: A549, HUVEC, Hepa1-6 and HepG2 whole cell lysates; mouse lung (7 days supernatant),

mouse placenta, rat placenta, rat lung. ICC/IF: Hepa1-6 cells. Flow Cyt (intra): Hepa1-6 cells.

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

Purity Protein A purified

Clonality Monoclonal
Clone number EPR17272-21

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab182973 in the following tested applications.

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

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Application	Abreviews	Notes
Flow Cyt (Intra)		1/60.
WB		1/1000. Detects a band of approximately 45 kDa (predicted molecular weight: 45 kDa).
ICC/IF		1/100.

Target

Function This inhibitor acts as 'bait' for tissue plasminogen activator, urokinase, and protein C. Its rapid

interaction with TPA may function as a major control point in the regulation of fibrinolysis.

Tissue specificity Found in plasma and platelets and in endothelial, hepatoma and fibrosarcoma cells.

Involvement in disease Defects in SERPINE1 are the cause of plasminogen activator inhibitor-1 deficiency (PAI-1D)

[MIM:613329]. It is a hematologic disorder characterized by increased bleeding after trauma, injury, or surgery. Affected females have menorrhagia. The bleeding defect is due to increased fibrinolysis of fibrin blood clots due to deficiency of plasminogen activator inhibitor-1, which

inhibits tissue and urinary activators of plasminogen.

Note=High concentrations of SERPINE1 seem to contribute to the development of venous but not

arterial occlusions.

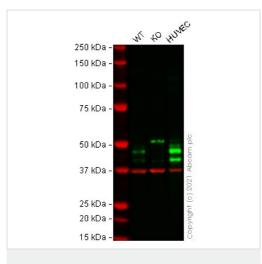
Sequence similarities Belongs to the serpin family.

Post-translational Inactivated by proteolytic attack of the urokinase-type (u-PA) and the tissue-type (TPA), cleaving

modifications the 369-Arg--Met-370 bond.

Cellular localization Secreted.

Images



Western blot - Anti-PAI1 antibody [EPR17272-21] (ab182973)

All lanes: Anti-PAI1 antibody [EPR17272-21] (ab182973) at

1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: SERPINE1 knockout A549 cell lysate

Lane 3: HUVEC cell lysate

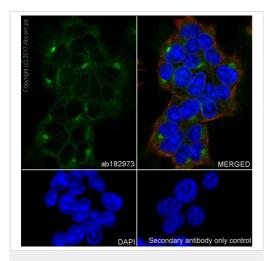
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 45 kDa **Observed band size:** 48 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab182973 observed at 48 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab182973 was shown to react with PAI1 in wild-type A549 cells in Western blot. The band observed in the edited lysate lane above 45 kDa is likely to represent SERPINE1 with an insertion. This has not been investigated further. Wild-type A549 and SERPINE1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween[®]) before incubation with ab182973 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.

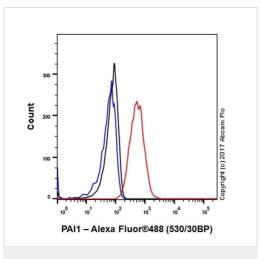


Immunocytochemistry/ Immunofluorescence - Anti-PAI1 antibody [EPR17272-21] (ab182973)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Hepa1-6 (mouse hepatoma epithelial cell line) cells labeling PAI1 with ab182973 at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on Hepa1-6 cells.

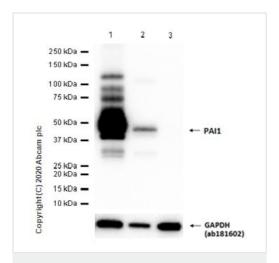
The nuclear counterstain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

-ve control: PBS only, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-PAI1 antibody [EPR17272-21] (ab182973)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methonol-permeabilized Hepa1-6 (mouse hepatoma epithelial cell line) cells labeling PAI1 with ab182973 at 1/60 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled 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.



Western blot - Anti-PAI1 antibody [EPR17272-21] (ab182973)

All lanes : Anti-PAI1 antibody [EPR17272-21] (ab182973) at 1/1000 dilution

Lane 1 : Rat placenta tissue lysate

Lane 2 : Rat lung tissue lysate
Lane 3 : Rat liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

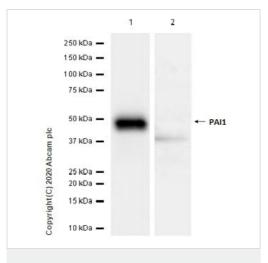
dilution

Predicted band size: 45 kDa **Observed band size:** 45 kDa

Exposure time: 20 seconds

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

The expression level of mouse and rat PAI1 may be low in normal liver tissue (PMID: 21898503). This antibody detects no bands in rat liver.



Western blot - Anti-PAI1 antibody [EPR17272-21] (ab182973)

All lanes : Anti-PAI1 antibody [EPR17272-21] (ab182973) at 1/1000 dilution

Lane 1: Mouse placenta tissue lysate

Lane 2: Mouse liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 45 kDa **Observed band size:** 45 kDa

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

The expression level of mouse and rat PAI1 may be low in normal liver tissue (PMID: 21898503). This antibody detects a 37kDa extra band without BOI in mouse liver.

Exposure time:

Lane 1: 3.25 seconds
Lane 2: 180 seconds

All lanes : Anti-PAI1 antibody [EPR17272-21] (ab182973) at 1/1000 dilution

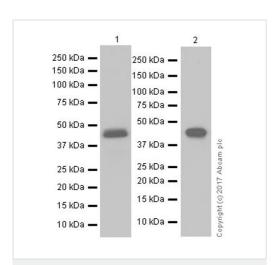
Lane 1 : Hepa1-6 (mouse hepatoma epithelial cell line) whole cell lysate

Lane 2 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

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



Western blot - Anti-PAI1 antibody [EPR17272-21] (ab182973)

Developed using the ECL technique.

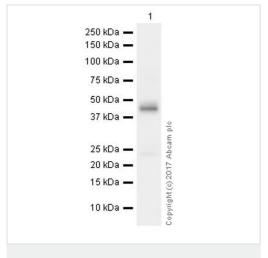
Predicted band size: 45 kDa **Observed band size:** 45 kDa

Blocking and dilution buffer: 5% NFDM/TBST

Exposure times.

Lane 1: 1 second

Lane 2: 3 seconds



Western blot - Anti-PAI1 antibody [EPR17272-21] (ab182973)

Anti-PAI1 antibody [EPR17272-21] (ab182973) at 1/1000 dilution + Mouse lung (7 days supernatant) at 10 μ l

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

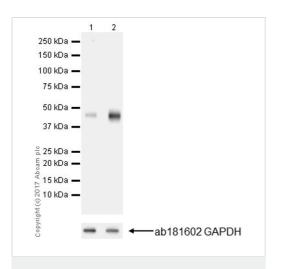
Developed using the ECL technique.

Predicted band size: 45 kDa **Observed band size:** 45 kDa

Exposure time: 3 seconds

Exposure time: 5 300

Blocking and dilution buffer: 5% NFDM/TBST



Western blot - Anti-PAI1 antibody [EPR17272-21] (ab182973)

All lanes : Anti-PAI1 antibody [EPR17272-21] (ab182973) at 1/5000 dilution

Lane 1: HepG2 (human hepatocellular carcinoma epithelial cell) grown in serum-free media for 18 hours, whole cell lysate

Lane 2: HepG2 grown in serum-free media for 18 hours, then treated with 10ng/ml TGFß for 24 hours, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

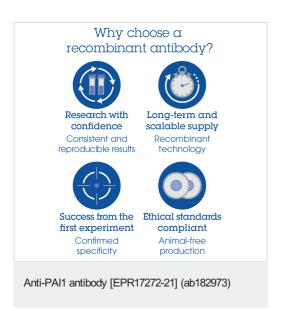
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 45 kDa **Observed band size:** 45 kDa

Exposure time: 180 seconds

Blocking and diluting buffer: 5% NFDM/TBST

This image was performed on a BIO-RAD[®] ChemiDoc[™] MP instrument. The expression profile observed is consistent with what has been described in the literature (PMID: 20519507).



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