abcam

Product datasheet

Anti-PAI1 antibody [EPR21850-82] ab222754

リコンビナント RabMAb

10 References 画像数 11

製品の概要

製品名	Anti-PAI1 antibody [EPR21850-82]
製品の詳細	Rabbit monoclonal [EPR21850-82] to PAI1
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), IP, ICC/IF, WB **適用なし:** IHC-P
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HepG2, HUVEC and Hepa1-6 whole cell lysate. Human, mouse and rat placenta lysate. Human liver lysate. Rat lung lysate. Serum starved NIH/3T3 treated with TGF beta1 supernatant lysate. Serum starved NIH/3T3 treated with TGF beta1 and Brefeldin A whole cell lysate. ICC/IF: HUVEC cells. Serum starved NIH/3T3 treated with TGF beta and Brefeldin A cells. Flow Cyt (intra): Serum starved NIH/3T3 treated with TGF beta and Brefeldin A cells, HUVEC cells. IP: HepG2 whole cell lysate.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR21850-82
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab222754の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/60.
IP		1/30.
ICC/IF		Use a concentration of 0.1 µg/ml. This product gave a positive signal in HUVEC (-ve: HEK293) fixed with 4% formaldehyde (10 min).
WB		1/1000. Predicted molecular weight: 45 kDa.

追加情報 Is unsuitable for IHC-P.

ターゲット情報

機能 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.

組織特異性 Found in plasma and platelets and in endothelial, hepatoma and fibrosarcoma cells.

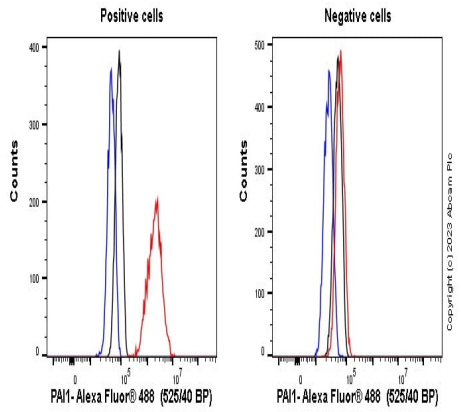
関連疾患 Defects in SERPINE1 are the cause of plasminogen activator inhibitor-1 deficiency (PAI-1D) [MIM:613329]. It is a hematologic disorder characterized by increased bleeding after trauma, injury, or surgery. Affected females have menorrhagia. The bleeding defect is due to increased fibrinolysis of fibrin 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.

配列類似性 Belongs to the serpin family.

翻訳後修飾 Inactivated by proteolytic attack of the urokinase-type (u-PA) and the tissue-type (TPA), cleaving the 369-Arg-
-Met-370 bond.

細胞内局在 Secreted.

画像



Flow Cytometry (Intracellular) - Anti-PAI1 antibody [EPR21850-82] (ab222754)

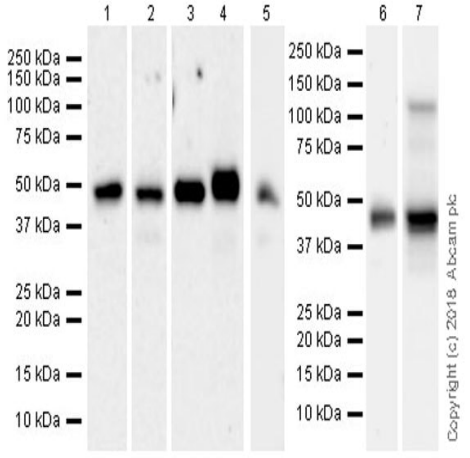
Flow cytometry overlay histogram showing left HUVEC positive cells and right negative HEK293 stained with ab222754 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab222754) (1x 10^6 in 100µl at 0.2µg/ml (1/9900)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in HUVEC Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Western blot - Anti-PAI1 antibody [EPR21850-82] (ab222754)

All lanes : Anti-PAI1 antibody [EPR21850-82] (ab222754) at 1/1000 dilution

Lane 1 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 2 : Human liver lysate
Lane 3 : Mouse placenta lysate
Lane 4 : Rat placenta lysate
Lane 5 : Hepa1-6 (Mouse hepatoma epithelial cell line) whole cell lysate
Lane 6 : Human placenta lysate
Lane 7 : HUVEC (Human umbilical vein endothelial cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
Lanes 1-5 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution
Lanes 6-7 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at

1/100000 dilution

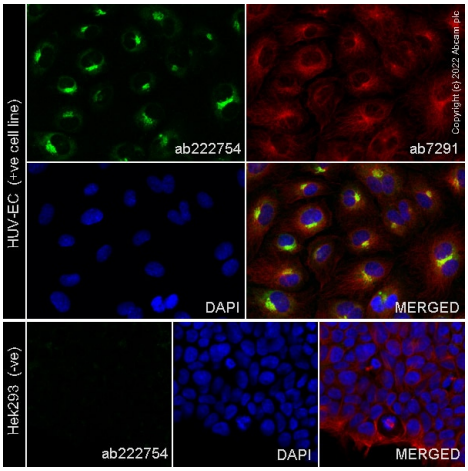
Predicted band size: 45 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1-4 and 6: 3 minutes; Lane 5: 37 seconds: Lane 7: 8 seconds.

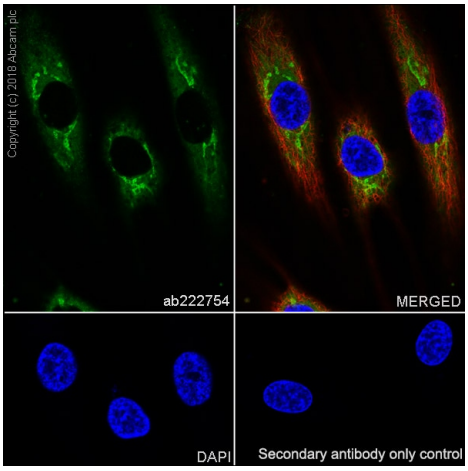
PAI1 forms complex with its target protease, t-PA (lane 7). The molecular mass observed is consistent with what has been described in the literature (PMID 21596853).

Lanes 6 and 7 were developed with a high sensitivity ECL substrate.



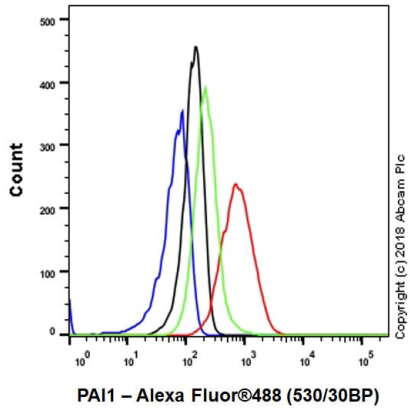
Immunocytochemistry/ Immunofluorescence - Anti-PAI1 antibody [EPR21850-82] (ab222754)

ab222754 staining SERPINE1 in HUV-EC cells, with negative expression in HEK293 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised with 0.1% Triton x-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab222754 at 0.1 µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin at 0.5 µg/ml. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150119**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 647), pre-adsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



Immunocytochemistry/ Immunofluorescence - Anti-PAI1 antibody [EPR21850-82] (ab222754)

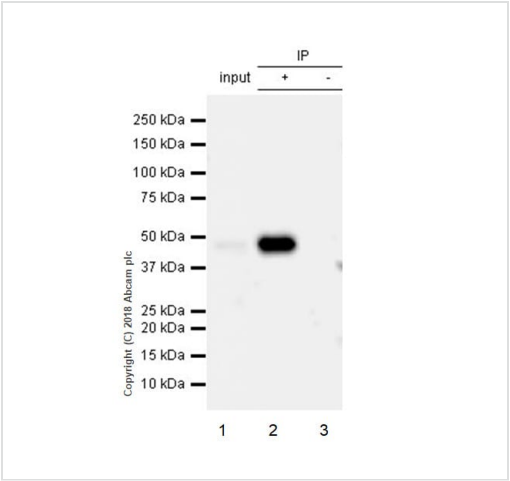
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HUVEC (Human umbilical vein endothelial cell line) cells labeling PAI1 with ab222754 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1,000 dilution (green). Confocal image showing cytoplasmic staining in HUVEC cell line. The nuclear counter stain 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-PAI1 antibody [EPR21850-82] (ab222754)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized NIH/3T3 cells serum-starved for 4 hours, treated with TGF-ß (10ng/ml) for 3 hours, and then with TGF-ß (10ng/ml) and BFA (300ng/ml) together for 18 hours (Red) / Untreated control (Green) labeling PAI1 with ab222754 at 1/60 (red/green) 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.

The expression of PAI-1 is induced by TGF-ß in NIH/3T3 cell line (PMID 17890327).



Immunoprecipitation - Anti-PAI1 antibody [EPR21850-82] (ab222754)

PAI1 was immunoprecipitated from 0.35 mg HepG2 (human hepatocellular carcinoma epithelial cell line) whole cell lysate with ab222754 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab222754 at 1/1,000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5,000 dilution.

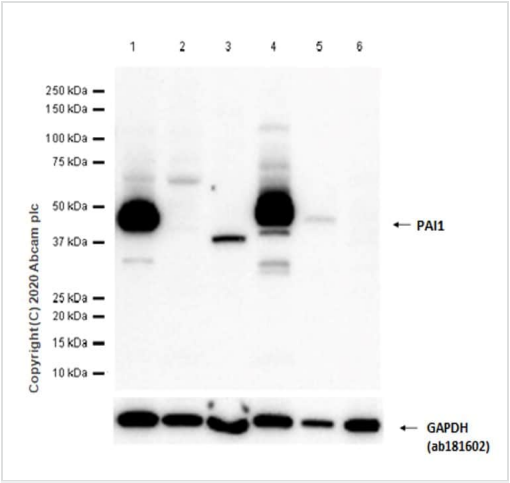
Lane 1: HepG2 whole cell lysate 10 µg (Input).

Lane 2: ab222754 IP in HepG2 whole cell lysate (+).

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab222754 in HepG2 whole cell lysate (-).

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-PAI1 antibody [EPR21850-82] (ab222754)

All lanes : Anti-HIF-1 alpha antibody [EPR16897] (**ab179483**) at 1/1000 dilution

Lane 1 : Mouse placenta tissue lysate

Lane 2 : Mouse lung tissue lysate

Lane 3 : Mouse liver tissue lysate

Lane 4 : Rat placenta tissue lysate

Lane 5 : Rat lung tissue lysate

Lane 6 : 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

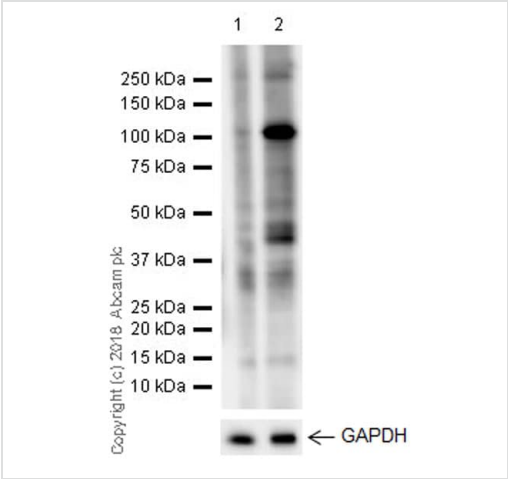
Additional bands at: 37 kDa (possible non-specific binding)

Exposure time: 3 minutes

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

The expression levels of mouse and rat PAI1 may be low in normal liver tissue (PMID: 21898503). This antibody detects a 37 kDa extra band and no specific band in mouse liver and no bands in rat liver.

Although lung tissue is reported to be PAI1 positive (PMID: 21768189, PMID: 17032919), this antibody can't detect band of target in mouse lung and detects weak target band in rat lung.



Western blot - Anti-PAI1 antibody [EPR21850-82] (ab222754)

All lanes : Anti-PAI1 antibody [EPR21850-82] (ab222754) at 1/1000 dilution

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast cell line) serum-starved for 4 hours, whole cell lysate
Lane 2 : NIH/3T3 serum-starved for 4 hours then treated with 10 ng/ml TGF ß1 (**ab50036**) for 3 hours, then with 10 ng/ml TGF ß1 (**ab50036**) and 300 ng/ml Brefeldin A (BFA) together for 18 hours, whole cell lysate

Lysates/proteins at 10 µg per lane.

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

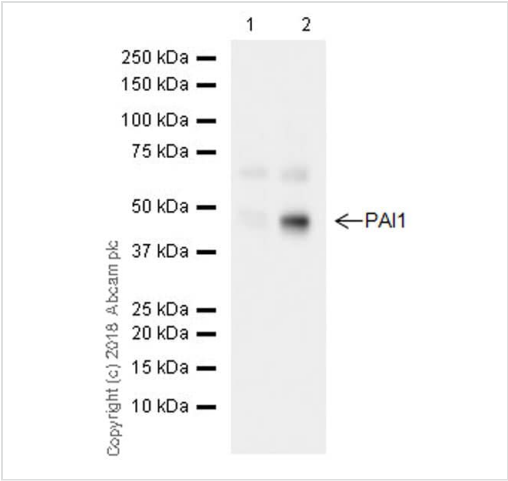
Predicted band size: 45 kDa

Exposure time: 32 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression of PAI-1 is induced by TGF-β in the NIH/3T3 cell line (PMID 17890327). The 110 kDa band likely represents PAI-1 in complex with its target protease, t-PA (PMID 21596853).

The blot was developed with a high sensitivity ECL substrate.



Western blot - Anti-PAI1 antibody [EPR21850-82] (ab222754)

All lanes : Anti-PAI1 antibody [EPR21850-82] (ab222754) at 1/1000 dilution

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast cell line) serum-starved for 18 hours, then collected the supernatant lysate
Lane 2 : NIH/3T3 serum-starved for 18 hours then treated with 10 ng/ml TGF ß1 (**ab50036**) for 24 hours, then collected the supernatant lysate

Lysates/proteins at 10 µg per lane.

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

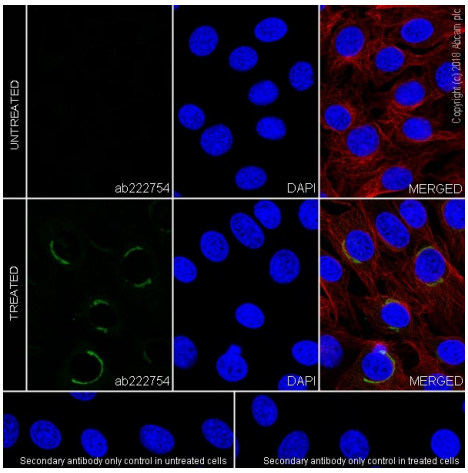
Predicted band size: 45 kDa

Exposure time: 26 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

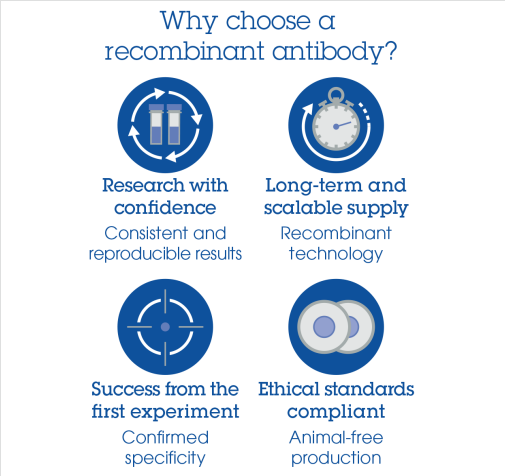
The expression of PAI-1 is induced by TGF-β in the NIH/3T3 cell line (PMID 17890327).

The blot was developed with a high sensitivity ECL substrate.



Immunocytochemistry/ Immunofluorescence - Anti-PAI1 antibody [EPR21850-82] (ab222754)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling PAI1 with ab222754 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1,000 dilution (green). Confocal image showing the signal is increased in 4 hour serum-starved NIH/3T3 cells treated with TGF-β (10 ng/ml) for 3 hours, then with TGF-β (10 ng/ml) and BFA (300 ng/ml) together for 18 hours. The expression of PAI-1 is induced by TGF-β in the NIH/3T3 cell line (PMID 17890327). The nuclear counter stain 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.



Anti-PAI1 antibody [EPR21850-82] (ab222754)

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