abcam

ab222754

Anti-PAI1 antibody [EPR21850-82]

Anti-PAI1 antibody [EPR21850-82] (ab222754) is a rabbit monoclonal antibody detecting PAI1 in **Western Blot, Flow Cytometry (Intra), IP, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 10 publications

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR21850-82
Purification technique	Affinity purification Protein A
Concentration	0.616 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human

Dilution info	1/30
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	0.1 µg/mL
Notes	This product gave a positive signal in HUVEC (-ve: HEK293) fixed with 4% formaldehyde (10 min).

Species	Human
Dilution info	0.1 µg/mL

Notes	This product gave a positive signal in HUVEC (-ve: HEK293) fixed with 4% formaldehyde (10 min).

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/60
Notes	-

Species	Human
Dilution info	1/60
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Not recommended

Species	Human
Dilution info	-
Notes	Tested with human, mouse, and rat kidney tissue.

Species	Mouse
Dilution info	-

Notes	Tested with human, mouse, and rat kidney tissue.

Species	Rat
Dilution info	-
Notes	Tested with human, mouse, and rat kidney tissue.

Target data

See full target information SERPINE1

Function	Serine protease inhibitor. Inhibits TMPRSS7 (PubMed:15853774). Is a primary inhibitor of tissue-type plasminogen activator (PLAT) and urokinase-type plasminogen activator (PLAU). As PLAT inhibitor, it is required for fibrinolysis down-regulation and is responsible for the controlled degradation of blood clots (PubMed:17912461, PubMed:8481516, PubMed:9207454, PubMed:21925150). As PLAU inhibitor, it is involved in the regulation of cell adhesion and spreading (PubMed:9175705). Acts as a regulator of cell migration, independently of its role as protease inhibitor (PubMed:15001579, PubMed:9168821). It is required for stimulation of keratinocyte migration during cutaneous injury repair (PubMed:18386027). It is involved in cellular and replicative senescence (PubMed:16862142). Plays a role in alveolar type 2 cells senescence in the lung (By similarity). Is involved in the regulation of cementogenic differentiation of periodontal ligament stem cells, and regulates odontoblast differentiation and dentin formation during odontogenesis (PubMed:25808697, PubMed:27046084).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-PAI1 antibody [EPR21850-82] (ab222754) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Mouse, Rat samples.

What is the molecular weight of PAI1?
Anti-PAI1 [EPR21850-82] (ab222754) specifically detects a band for PAI1 (UniProt: P05121) at a molecular weight of 45kDa.

Trusted by the scientific community
Anti-PAI1 [EPR21850-82] (ab222754) was first used in a scientific publication in 2018 and has been cited over 10 times in peer-reviewed journals.

Other related products
We have a range of other formats of antibody clone [EPR21850-82] also available for your convenience: ab222754, Carrier free - ab237780, Alexa Fluor® 647 - ab303581, Alexa Fluor® 488 - ab307393, PE - ab314276

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

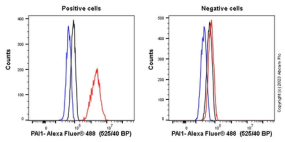
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

11 product images



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

PAI1 Flow Cytometry (Intracellular) staining using rabbit Anti-PAI1 antibody

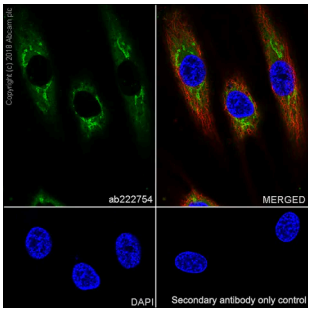
Flow cytometry overlay histogram showing left HUVEC positive cells and right negative HEK293 stained with ab222754 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab222754) (1x 106 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.

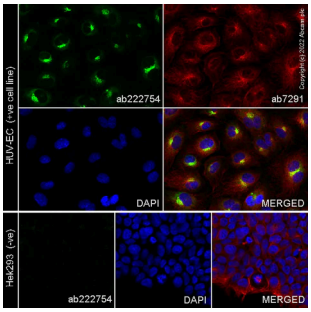
This antibody gave a positive signal in HUVEC Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



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

PAI1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PAI1 antibody

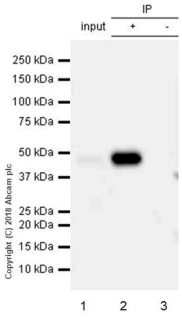
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HUVEC (Human umbilical vein endothelial cell line) cells labeling PAI1 with ab222754 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1,000 dilution (green). 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.



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

PAI1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PAI1 antibody

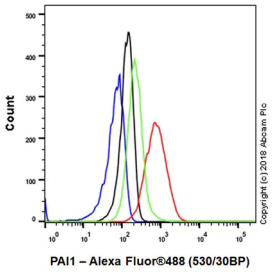
ab222754 staining SERPINE1 in HUV-EC cells, with negative expression in HEK293 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised with 0.1% Triton x-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated 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.



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.
All lanes:
Immunoprecipitation - Anti-PAI1 antibody [EPR21850-82] (ab222754)

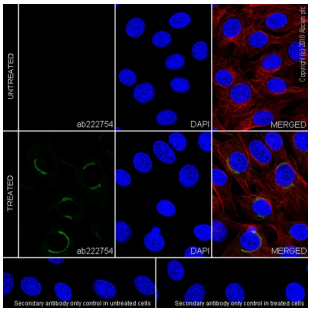
Predicted band size: 45 kDa



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

PAI1 Flow Cytometry (Intracellular) staining using rabbit Anti-PAI1 antibody

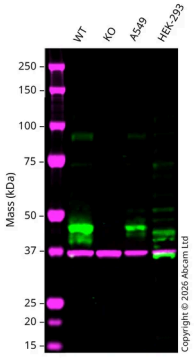
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized NIH/3T3 cells serum-starved for 4 hours, treated with TGF-ß (10ng/ml) for 3 hours, and then with TGF-ß (10ng/ml) and BFA (300ng/ml) together for 18 hours (Red) / Untreated control (Green) labeling PAI1 with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



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

PAI1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PAI1 antibody

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.



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

PAI1 Western blot staining using rabbit Anti-PAI1 antibody

Western blot: Anti-PAI1 antibody [EPR21850-82] ab222754 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 45 kDa in Wild-type U-87 MG cell lysates with no signal observed at this size in SERPINE1 knockout U-87 MG cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-PAI1 antibody [EPR21850-82] (ab222754) at 1/1000 dilution

Lane 1:
Wild-type U-87 MG at 20 µg

Lane 2:
Western blot - Human SERPINE1 knockout U-87 MG cell line (ab306851) at 20 µg

Lane 3:
A549 at 20 µg

Lane 4:
HEK-293 at 20 µg

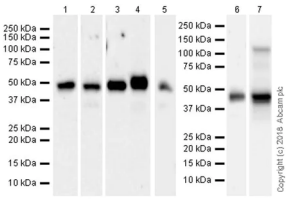
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 45 kDa

Observed band size: 45 kDa



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

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.

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

Lane 1:
HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate at 20 µg

Lane 2:
Human liver lysate at 20 µg

Lane 3:
Mouse placenta lysate at 20 µg

Lane 4:
Rat placenta lysate at 20 µg

Lane 5:
Hepa1-6 (Mouse hepatoma epithelial cell line) whole cell lysate at 20 µg

Lane 6:
Human placenta lysate at 20 µg

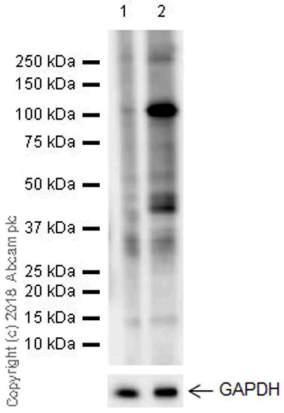
Lane 7:
HUVEC (Human umbilical vein endothelial cell line) whole cell lysate at 20 µg

Secondary

Lanes 1 - 5:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Lanes 6 - 7:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 45 kDa



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

PAI1 Western blot staining using rabbit Anti-PAI1 antibody

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.

All lanes:
Western blot - 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 at 10 µg

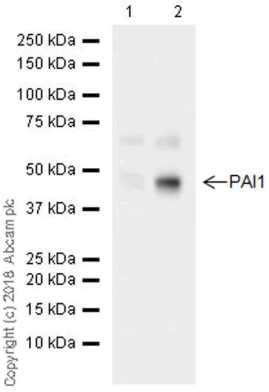
Lane 2:
NIH/3T3 serum-starved for 4 hours then treated with 10 ng/ml TGF β1 (ab50036) for 3 hours, then with 10 ng/ml TGF β1 (ab50036) and 300 ng/ml Brefeldin A (BFA) together for 18 hours, whole cell lysate at 10 µg

Secondary

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

Predicted band size: 45 kDa

Exposure time: 32s



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

PAI1 Western blot staining using rabbit Anti-PAI1 antibody

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.

All lanes:
Western blot - 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 at 10 µg

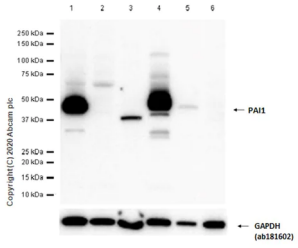
Lane 2:
NIH/3T3 serum-starved for 18 hours then treated with 10 ng/ml TGF β1 (ab50036) for 24 hours, then collected the supernatant lysate at 10 µg

Secondary

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

Predicted band size: 45 kDa

Exposure time: 26s



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

PAI1 Western blot staining using rabbit Anti-PAI1 antibody

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.

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

Lane 1:

Mouse placenta tissue lysate at 20 µg

Lane 2:
Mouse lung tissue lysate at 20 µg

Lane 3:
Mouse liver tissue lysate at 20 µg

Lane 4:
Rat placenta tissue lysate at 20 µg

Lane 5:
Rat lung tissue lysate at 20 µg

Lane 6:
Rat liver tissue lysate at 20 µg

Secondary

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

Predicted band size: 45 kDa

Observed band size: 37 kDa, 45 kDa

Exposure time: 3min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.