abcam

Product datasheet

Anti-PAI1 antibody [EPR17795] - BSA and Azide free ab250924



9 Images

Overview

Product name Anti-PAI1 antibody [EPR17795] - BSA and Azide free

Description Rabbit monoclonal [EPR17795] to PAI1 - BSA and Azide free

Host species Rabbit

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

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: A549, HepG2 and HUVEC cell lysates; Human fetal liver and spleen lysates; Human PAI1 full

length protein. Flow Cyt (intra): HepG2 cells. ICC/IF: HepG2 cells. IP: HepG2 cells.

General notes ab250924 is the carrier-free version of ab187262.

> 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 cellbased 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**.

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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

ClonalityMonoclonalClone numberEPR17795

Isotype IgG

Applications

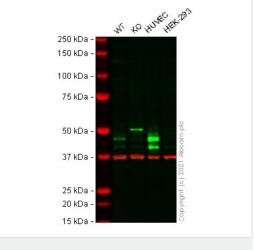
The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab250924 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
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 45 kDa (predicted molecular weight: 45 kDa).
ICC/IF		Use at an assay dependent concentration.

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 modifications	Inactivated by proteolytic attack of the urokinase-type (u-PA) and the tissue-type (TPA), cleaving the 369-ArgMet-370 bond.	
Cellular localization	Secreted.	



Western blot - Anti-PAI1 antibody [EPR17795] - BSA and Azide free (ab250924)

All lanes : Anti-PAI1 antibody [EPR17795] (ab187262) at 1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: SERPINE1 knockout A549 cell lysate

Lane 3: HUVEC cell lysate
Lane 4: HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

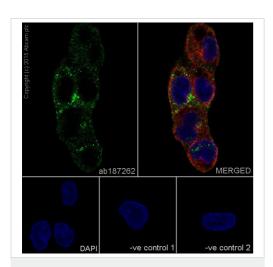
Performed under reducing conditions.

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

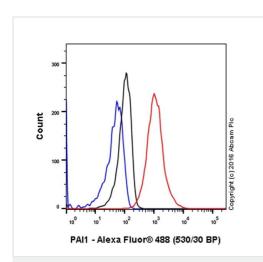
This data was developed using the same antibody clone in a different buffer formulation (<u>ab187262</u>).

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab187262</u> observed at 48 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab187262 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 ab187262 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 [EPR17795] - BSA and Azide free (ab250924)



Flow Cytometry (Intracellular) - Anti-PAI1 antibody [EPR17795] - BSA and Azide free (ab250924)

This data was developed using <u>ab187262</u>, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma) cells labeling PAI1 with <u>ab187262</u> at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (<u>ab150077</u>) secondary antibody at 1/400 dilution (green). Cytoplasm staining on HepG2 cells was observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red). The negative controls are as follows:

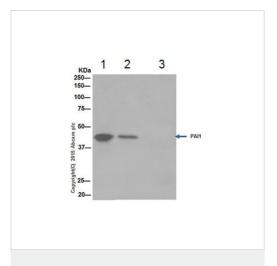
-ve control 1: <u>ab187262</u> at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/400 dilution.

This data was developed using <u>ab187262</u>, the same antibody clone in a different buffer formulation.

ab187262 staining PAI1 in the human cell line HepG2 (human hepatocellular carcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/60. A goat anti rabbit lgG (Alexa Fluor[®] 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)

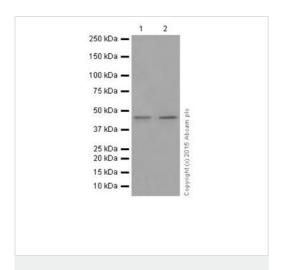


Immunoprecipitation - Anti-PAl1 antibody

[EPR17795] - BSA and Azide free (ab250924)

This data was developed using <u>ab187262</u>, the same antibody clone in a different buffer formulation.PAI1 was immunoprecipitated from 1mg of HepG2 (Human liver hepatocellular carcinoma) whole cell lysate with <u>ab187262</u> at 1/40 dilution. Western blot was performed from the immunoprecipitate using <u>ab187262</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/1500 dilution. Lane 1: HepG2 whole cell lysate 10 µg (Input). Lane 2: <u>ab187262</u> IP in HepG2 whole cell lysate. Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab187262</u> in HepG2 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 30 seconds.



Western blot - Anti-PAI1 antibody [EPR17795] - BSA and Azide free (ab250924)

All lanes : Anti-PAI1 antibody [EPR17795] (<u>ab187262</u>) at 1/1000 dilution

Lane 1 : Human fetal liver lysate

Lane 2 : Human fetal spleen lysate

Lysates/proteins at 10 µg per lane.

Secondary

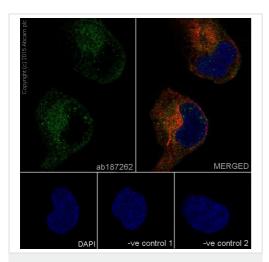
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

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

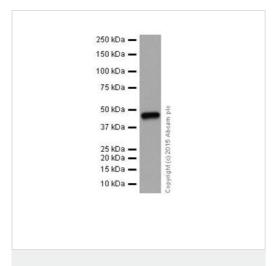
Exposure time: 3 minutes

This data was developed using <u>ab187262</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-PAI1 antibody [EPR17795] - BSA and Azide free (ab250924)



Western blot - Anti-PAI1 antibody [EPR17795] - BSA and Azide free (ab250924)

This data was developed using <u>ab187262</u>, the same antibody clone in a different buffer formulation.lmmunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HT1080 (Human fibrosarcoma cells) cells labeling PAI1 with <u>ab187262</u> at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (<u>ab150077</u>) secondary antibody at 1/400 dilution (green). Cytoplasm staining on HT1080 cells was observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red). The negative controls are as follows:

-ve control 1: <u>ab187262</u> at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution

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Anti-PAI1 antibody [EPR17795] (ab187262) at 1/5000 dilution + HepG2 (Human liver hepatocellular carcinoma) whole cell lysate at 20 μg

Secondary

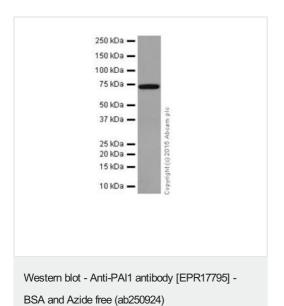
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

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

Exposure time: 1 minute

This data was developed using <u>ab187262</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Anti-PAI1 antibody [EPR17795] ($\underline{ab187262}$) at 1/5000 dilution + Human PAI1 full length protein at 0.01 μg

Secondary

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 45 kDa **Observed band size:** 70 kDa

Exposure time: 30 seconds

Exposure time. 30 seconds

This data was developed using <u>ab187262</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



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