abcam

Product datasheet

Anti-AACT antibody [EPR17088-68] ab205198

Recombinant RabMAb

2 References 9 Images

Overview

Product name Anti-AACT antibody [EPR17088-68]

Description Rabbit monoclonal [EPR17088-68] to AACT

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, IP

Species reactivity Reacts with: Human

Immunogen Full length native protein (purified). This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human plasma (untreated and PNGase F treated) and blood; Human fetal spleen lysate.

IHC-P: Human tonsil tissue. ICC/IF: Raji cells. IP: Human plasma 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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR17088-68

Isotype lgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab205198 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
WB		1/1000. Detects a band of approximately 50-70 kDa (predicted molecular weight: 47 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/50.
IP		1/40.

Target

Function Although its physiological function is unclear, it can inhibit neutrophil cathepsin G and mast cell

chymase, both of which can convert angiotensin-1 to the active angiotensin-2.

Tissue specificity Plasma. Synthesized in the liver. Like the related alpha-1-antitrypsin, its concentration increases

in the acute phase of inflammation or infection. Found in the amyloid plaques from the

hippocampus of Alzheimer disease brains.

Involvement in disease Defects in SERPINA3 may be a cause of chronic obstructive pulmonary disease (COPD)

[MIM:107280].

Sequence similarities Belongs to the serpin family.

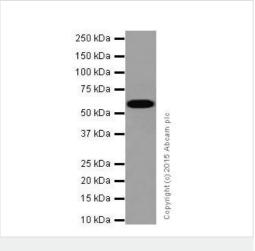
Domain The reactive center loop (RCL) extends out from the body of the protein and directs binding to the

target protease. The protease cleaves the serpin at the reactive site within the RCL, establishing a covalent linkage between the carboxyl group of the serpin reactive site and the serine hydroxyl

of the protease. The resulting inactive serpin-protease complex is highly stable.

Cellular localization Secreted.

Images



Western blot - Anti-AACT antibody [EPR17088-68] (ab205198)

Anti-AACT antibody [EPR17088-68] (ab205198) at 1/2000 dilution + Human plasma at 10 µg

Secondary

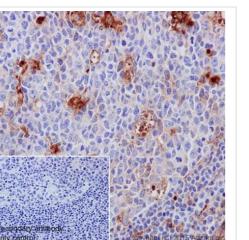
Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/10000 dilution

Predicted band size: 47 kDa **Observed band size:** 50-70 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID: 9880565).

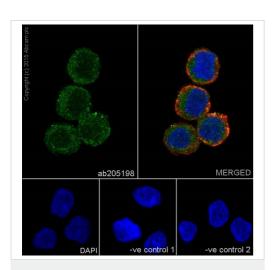


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-AACT antibody
[EPR17088-68] (ab205198)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling AACT with ab205198 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab205198) at 1/500 dilution. Cytoplasmic staining on human tonsil tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



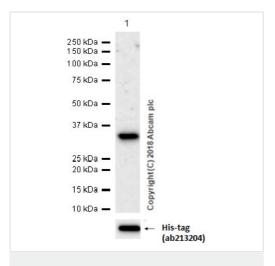
Immunocytochemistry/ Immunofluorescence - Anti-AACT antibody [EPR17088-68] (ab205198)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Raji (Human Burkitt's lymphoma cell line) cells labeling AACT with ab205198 at 1/50 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic and weak nuclear staining on Raji cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (Alexa Fluor[®] 594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab205198 at 1/50 dilution followed by <u>ab150120</u> (Alexa Fluor[®] 594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor[®] 488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



Western blot - Anti-AACT antibody [EPR17088-68] (ab205198)

Anti-AACT antibody [EPR17088-68] (ab205198) at 1/1000 dilution + His-Tagged Human SERPINA3 (aa 24 to 324) recombinant protein 100ng

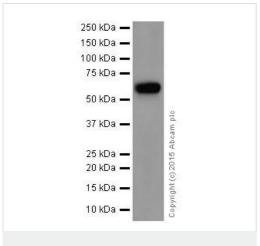
Secondary

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

Predicted band size: 47 kDa **Observed band size:** 33 kDa

Exposure time: 3 minutes

Blocking/Diluting buffer and concentration 5% NFDM/TBST



Western blot - Anti-AACT antibody [EPR17088-68] (ab205198)

Anti-AACT antibody [EPR17088-68] (ab205198) at 1/1000 dilution + Human blood at 10 μg

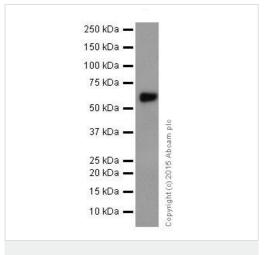
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 47 kDa **Observed band size:** 50-70 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-AACT antibody [EPR17088-68] (ab205198)

Anti-AACT antibody [EPR17088-68] (ab205198) at 1/1000 dilution + Human fetal spleen lysate at 10 μg

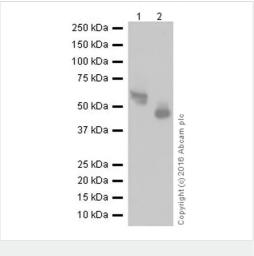
Secondary

Anti-Rabbit $\lg G$ (HRP), specific to the non-reduced form of $\lg G$ at 1/10000 dilution

Predicted band size: 47 kDa **Observed band size:** 50-70 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-AACT antibody [EPR17088-68] (ab205198)

All lanes : Anti-AACT antibody [EPR17088-68] (ab205198) at 1/2000 dilution

Lane 1: Untreated Human plasma

Lane 2: Human plasma treated with PNGase F

Lysates/proteins at 10 µg per lane.

Secondary

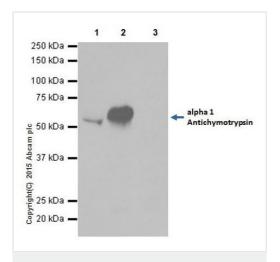
All lanes : Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/100000 dilution

Predicted band size: 47 kDa **Observed band size:** 50-70 kDa

Exposure time: 4 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

De-glycosylation treatment with PNGase, demonstrates a molecular weight shift from 50-70kDa to 46kDa. This result is consistent with what has been described in literature the literature (PMID:) 9880565.



Immunoprecipitation - Anti-AACT antibody [EPR17088-68] (ab205198)

AACT was immunoprecipitated from 1 mg of Human plasma lysate with ab205198 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab205198 at 1/1000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG was used as secondary antibody at 1/1500 dilution.

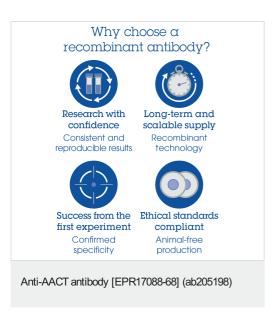
Lane 1: Human plasma lysate, 10 µg (Input).

Lane 2: ab205198 IP in Human plasma lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab205198 in Human plasma lysate.

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

Exposure time: 30 seconds.



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