abcam

Product datasheet

Anti-Thrombomodulin antibody [EPR18217-209] ab230010



2 References 9 Images

Overview

Product name Anti-Thrombomodulin antibody [EPR18217-209]

Description Rabbit monoclonal [EPR18217-209] to Thrombomodulin

Host species Rabbit

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

Species reactivity Reacts with: Mouse

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

Positive control WB: Mouse lung and placenta tissue lysates; bEND.3 whole cell lysate. IHC-P: Mouse lung and

stomach tissues. IHC-Fr: Mouse embryo E14.5 (developing lung) tissue. ICC/IF: bEND.3 cells.

Flow Cyt: bEND.3 cells. IP: bEND.3 whole cell lysate; Mouse lung tissue lysate.

General notesThis product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

Improved sensitivity and specificityLong-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: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

1

Clone number EPR18217-209

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise quarantee** covers the use of ab230010 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
IHC-Fr		1/500. Perform heat mediated antigen retrieval by using sodium citrate buffer (10 mM citrate pH 6.0 + 0.05% Tween-20).
ICC/IF		1/100.
IP		1/30.
Flow Cyt		1/500.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 75,105 kDa (predicted molecular weight: 62 kDa).

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Function

Thrombomodulin is a specific endothelial cell receptor that forms a 1:1 stoichiometric complex with thrombin. This complex is responsible for the conversion of protein C to the activated protein C (protein Ca). Once evolved, protein Ca scissions the activated cofactors of the coagulation mechanism, factor Va and factor VIIIa, and thereby reduces the amount of thrombin generated.

Tissue specificity

Endothelial cells are unique in synthesizing thrombomodulin.

Involvement in disease

Defects in THBD are the cause of thrombophilia due to thrombomodulin defect (THR-THBD) [MIM:188040]. A hemostatic disorder characterized by a tendency to thrombosis.

Defects in THBD are a cause of susceptibility to hemolytic uremic syndrome atypical type 6 (AHUS6) [MIM:612926]. An atypical form of hemolytic uremic syndrome. It is a complex genetic disease characterized by microangiopathic hemolytic anemia, thrombocytopenia, renal failure and absence of episodes of enterocolitis and diarrhea. In contrast to typical hemolytic uremic syndrome, atypical forms have a poorer prognosis, with higher death rates and frequent progression to end-stage renal disease. Note=Susceptibility to the development of atypical hemolytic uremic syndrome can be conferred by mutations in various components of or regulatory factors in the complement cascade system. Other genes may play a role in modifying the phenotype.

Sequence similarities

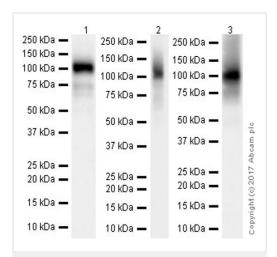
Contains 1 C-type lectin domain. Contains 6 EGF-like domains.

Post-translational modifications

N-glycosylated.

The iron and 2-oxoglutarate dependent 3-hydroxylation of aspartate and asparagine is (R)

Images



Western blot - Anti-Thrombomodulin antibody [EPR18217-209] (ab230010) **All lanes :** Anti-Thrombomodulin antibody [EPR18217-209] (ab230010) at 1/1000 dilution

Lane 1: Mouse lung tissue lysate at 20 µg

 $\textbf{Lane 2:} \ \mathsf{bEND.3} \ (\mathsf{mouse} \ \mathsf{brain} \ \mathsf{endothelioma} \ \mathsf{cell} \ \mathsf{line}) \ \mathsf{whole} \ \mathsf{cell}$

lysate at 10 µg

Lane 3: Mouse placenta tissue lysate at 10 µg

Secondary

 $\textbf{Lane 1:} \ \ \textbf{Goat Anti-Rabbit IgG H\&L (HRP)} \ (\underline{\textbf{ab97051}}) \ \ \textbf{at 1/100000}$

dilution

Lanes 2-3: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at

1/50000 dilution

Developed using the ECL technique.

Predicted band size: 62 kDa **Observed band size:** 105,75 kDa

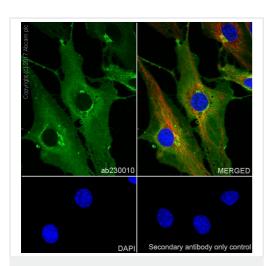
Exposure times: Lane 1: 1 minute; Lane 2: 20 seconds; Lane 3:

10 seconds.

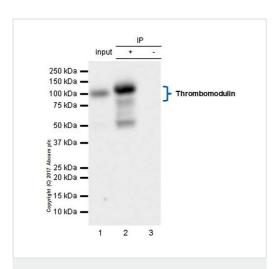
Blocking/Dilution buffer: 5% NFDM/TBST.

The blot was developed on a BIO-RAD® ChemiDoc™ MP

instrument.



Immunocytochemistry/ Immunofluorescence - Anti-Thrombomodulin antibody [EPR18217-209] (ab230010)



Immunoprecipitation - Anti-Thrombomodulin antibody [EPR18217-209] (ab230010)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized bEND.3 (mouse brain endothelioma cell line) cells labeling Thrombomodulin with ab230010 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 and membranous staining in bEND.3 cell line (PMID: 7622601; PMID: 8223719).

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).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution.

Thrombomodulin was immunoprecipitated from 0.35 mg of mouse lung tissue lysate with ab230010 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab230010 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

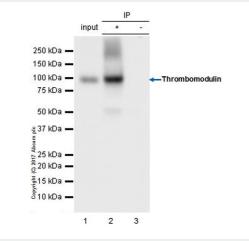
Lane 1: Mouse lung tissue lysate 10 µg (Input).

Lane 2: ab230010 IP in mouse lung tissue lysate.

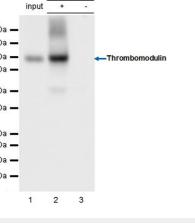
Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab230010 in mouse lung tissue lysate.

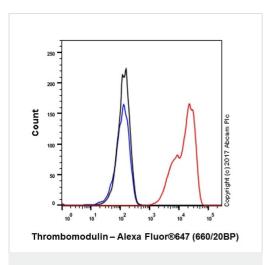
Exposure time: 10 seconds.

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



Immunoprecipitation - Anti-Thrombomodulin antibody [EPR18217-209] (ab230010)





Flow Cytometry - Anti-Thrombomodulin antibody [EPR18217-209] (ab230010)

Thrombomodulin was immunoprecipitated from 0.35 mg of bEND.3 (mouse brain endothelioma cell line) whole cell lysate with ab230010 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab230010 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Lane 1: bEND.3 whole cell lysate 10 µg (Input).

Lane 2: ab230010 IP in bEND.3 whole cell lysate.

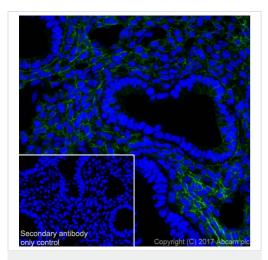
Lane 3: Rabbit monoclonal lgG (ab172730) instead of ab230010 in bEND.3 whole cell lysate.

Exposure time: 10 seconds.

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

Flow cytometric analysis of bEND.3 (mouse brain endothelioma cell line) cells labeling Thrombomodulin with ab230010 at 1/500 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.

Gated on total viable cells.

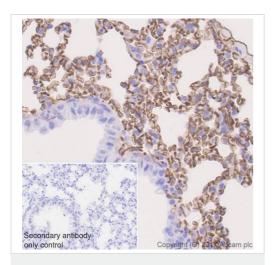


Immunohistochemistry (Frozen sections) - Anti-Thrombomodulin antibody [EPR18217-209] (ab230010)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse embryo E14.5 (developing lung) tissue labeling Thrombomodulin with ab230010 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution (green). Positive membrane staining in the developing lung in mouse E14.5 embryo (PMID: 28306049) is observed.

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.

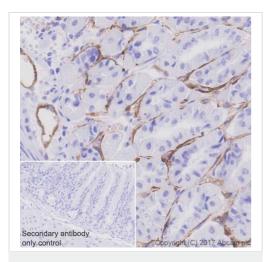


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Thrombomodulin antibody [EPR18217-209] (ab230010)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling Thrombomodulin with ab230010 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining on endothelial cells of mouse lung (PMID: 23946288; PMID: 10231031) 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) Ready to use.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Thrombomodulin antibody [EPR18217-209] (ab230010)

Immunohistochemical analysis of paraffin-embedded mouse stomach tissue labeling Thrombomodulin with ab230010 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining on endothelial cells of mouse stomach (PMID: 23946288; PMID: 10231031) 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) Ready to use.

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



(ab230010)

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