# abcam

# Product datasheet

# Anti-Thrombomodulin antibody [EPR18217-209] - BSA and Azide free ab230152



RabMAb

# 8 Images

#### Overview

Product name Anti-Thrombomodulin antibody [EPR18217-209] - BSA and Azide free

**Description** Rabbit monoclonal [EPR18217-209] to Thrombomodulin - BSA and Azide free

Host species Rabbit

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

Species reactivity Reacts with: Mouse

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

Positive control IHC-P: Mouse lung tissue.

**General notes** ab230152 is the carrier-free version of <u>ab230010</u>.

Our <u>carrier-free</u> 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 cell-based 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **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

Purity Protein A purified

**Clonality** Monoclonal

Clone number EPR18217-209

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab230152 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		Use at an assay dependent concentration. Detects a band of approximately 75,105 kDa (predicted molecular weight: 62 kDa).
IHC-Fr		Use at an assay dependent concentration.  Perform heat mediated antigen retrieval by using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

## **Target**

**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.

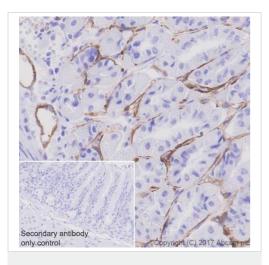
**Post-translational** N-glycosylated.

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

stereospecific within EGF domains.

**Cellular localization** Membrane.

#### **Images**



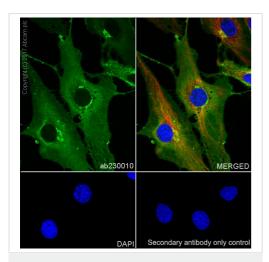
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Thrombomodulin antibody [EPR18217-209] - BSA and Azide free (ab230152)

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.

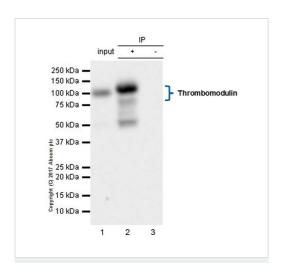
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230010).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Thrombomodulin antibody [EPR18217-209] - BSA and Azide free (ab230152)



Immunoprecipitation - Anti-Thrombomodulin antibody
[EPR18217-209] - BSA and Azide free (ab230152)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized bEND.3 (mouse brain endothelioma cell line) cells labeling Thrombomodulin with <a href="mailto:ab230010">ab230010</a> at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (<a href="mailto:ab150077">ab150077</a>) 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<sup>®</sup> 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<sup>®</sup> 488) (**ab150077**) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230010).

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

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

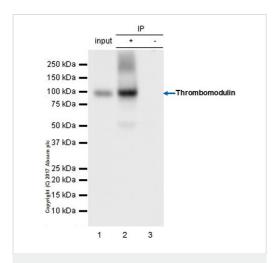
Lane 2: <u>ab230010</u> IP in mouse lung tissue lysate.

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

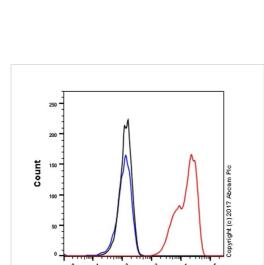
Exposure time: 10 seconds.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230010).



Immunoprecipitation - Anti-Thrombomodulin antibody [EPR18217-209] - BSA and Azide free (ab230152)



Flow Cytometry - Anti-Thrombomodulin antibody [EPR18217-209] - BSA and Azide free (ab230152)

Thrombomodulin - Alexa Fluor®647 (660/20BP)

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 IgG (<u>ab172730</u>) instead of <u>ab230010</u> in bEND.3 whole cell lysate.

Exposure time: 10 seconds.

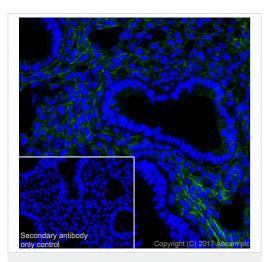
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab230010</u>).

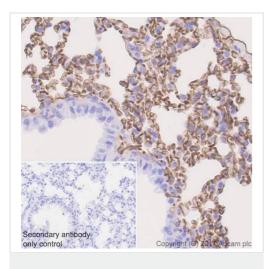
Flow cytometric analysis of bEND.3 (mouse brain endothelioma cell line) cells labeling Thrombomodulin with <u>ab230010</u> at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>), at 1/2000 dilution was used as the secondary antibody.

Gated on total viable cells.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab230010</u>).



Immunohistochemistry (Frozen sections) - Anti-Thrombomodulin antibody [EPR18217-209] - BSA and Azide free (ab230152)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Thrombomodulin antibody

[EPR18217-209] - BSA and Azide free (ab230152)

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 lgG H&L (Alexa Fluor<sup>®</sup> 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<sup>®</sup> 488) (ab150077) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230010).

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling Thrombomodulin with <u>ab230010</u> 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab230010).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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