**Overview**

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-Thrombospondin 1 antibody [EPR22927-54]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit monoclonal [EPR22927-54] to Thrombospondin 1</td>
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<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: WB, IHC-P, ICC/IF, Flow Cyt, IP</td>
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<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Human</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Recombinant fragment within Mouse Thrombospondin 1 aa 500 to the C-terminus. The exact sequence is proprietary. Database link: P35441</td>
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<tr>
<td><strong>Positive control</strong></td>
<td>WB: 3T3-L1 starved with 0.4% serum for 24 hours, then cultivated with 15% serum for 6 hours, whole cell lysate. HUVEC and mouse platelet lysates. IHC-P: Human spleen, human bone marrow and mouse spleen tissues. ICC/IF: HUVEC cells. Flow Cyt: HUVEC and 3T3-L1 cells. IP: HUVEC and mouse platelets lysate.</td>
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| **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**

<table>
<thead>
<tr>
<th><strong>Form</strong></th>
<th>Liquid</th>
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<tbody>
<tr>
<td><strong>Storage instructions</strong></td>
<td>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.</td>
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</tbody>
</table>
| **Storage buffer** | Preservative: 0.01% Sodium azide  
Constituents: PBS, 0.05% BSA, 40% Glycerol |
Purity: Protein A purified
Clonality: Monoclonal
Clone number: EPR22927-54
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab267388 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>1/1000. Predicted molecular weight: 129 kDa.</td>
<td></td>
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<tr>
<td>IHC-P</td>
<td>1/5000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.</td>
<td></td>
</tr>
<tr>
<td>ICC/IF</td>
<td>1/100.</td>
<td></td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>1/50.</td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>1/30.</td>
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</table>

Target

Relevance: Thrombospondin is a regulator of many biological processes including cell growth, adhesion, migration, platelet aggregation, and fibrin deposition and lysis. It interacts with a number of plasma proteins such as fibrinogen and plasminogen and co-polymerizes with fibrin in clot formation. Thrombospondin also has multiple binding sites that interact with molecules such as fibronectin, collagens, laminin and heparan sulphate proteoglycans as well as binding growth factors such as TGF-beta1. Thrombospondin exerts an anti-adhesive effect which leads to cell rounding and detachment.

Images
All lanes: Anti-Thrombospondin 1 antibody [EPR22927-54] (ab267388) at 1/1000 dilution

Lane 1: HUVEC (human umbilical vein endothelial cell) whole cell lysate
Lane 2: Mouse platelet lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 129 kDa

Exposure time: 3 minutes

The full-length TSP 1 (180kDa) and a 160-kDa band, likely to be an TSP 1 isoform or fragment, are observed.

The molecular weight observed is consistent with what has been described in the literature (PMID:1426766, 27588705).

Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes: Anti-Thrombospondin 1 antibody [EPR22927-54] (ab267388) at 1/1000 dilution

Lane 1: 3T3-L1 (mouse embryonic fibroblast) starved with 0.4% serum for 30 hours whole cell lysate
Lane 2: 3T3-L1 starved with 0.4% serum for 24 hours, then cultivated with 15% serum for 6 hours. whole cell lysate

Lysates/proteins at 10 µg per lane.

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

Predicted band size: 129 kDa

Exposure time: 3 minutes
TSP 1 is a serum-responsive gene. Its expression is elevated in quiescent 3T3 with serum addition.

The full-length TSP 1 (180kDa) and a 160-kDa band, likely to be an TSP 1 isoform or fragment, are observed.

The molecular weight observed is consistent with what has been described in the literature (PMID:1426766, 27588705).

Blocking/Dilution buffer: 5% NFDM/TBST.

Thrombospondin 1 was immunoprecipitated from 0.35 mg mouse platelets whole cell lysate 10µg with ab267388 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab267388 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

**Lane 1:** Mouse platelets whole cell lysate 10µg.

**Lane 2:** ab267388 IP in mouse platelets whole cell lysate.

**Lane 3:** Rabbit monoclonal IgG (ab172730) instead of ab267388 in mouse platelets whole cell lysate.

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

Exposure time: 8 seconds.

Thrombospondin 1 was immunoprecipitated from 0.35 mg HUVEC (human umbilical vein endothelial cell) whole cell lysate 10µg with ab267388 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab267388 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

**Lane 1:** HUVEC whole cell lysate 10µg.

**Lane 2:** ab267388 IP in HUVEC whole cell lysate.

**Lane 3:** Rabbit monoclonal IgG (ab172730) instead of ab267388 in HUVEC whole cell lysate.

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

Exposure time: 8 seconds.
Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling Thrombospondin 1 with ab267388 at 1/5000 dilution (0.1 µg/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer). Positive staining on the megakaryocytes and platelets in the mouse spleen is observed. The section was incubated with ab267388 for 10 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

Immunohistochemical analysis of paraffin-embedded human bone marrow tissue labeling Thrombospondin 1 with ab267388 at 1/5000 dilution (0.1 µg/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer). Positive staining on the megakaryocytes in the human bone marrow (PMID: 28239144). The section was incubated with ab267388 for 10 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.
Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling Thrombospondin 1 with ab267388 at 1/5000 dilution (0.1 µg/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer). Positive staining on the platelets in the human spleen (PMID: 28239144). The section was incubated with ab267388 for 10 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP Polymer).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

Immunofluorescent analysis of 100% methanol-fixed, permeabilized HUVEC (human umbilical vein endothelial cell) cells labeling Thrombospondin 1 with ab267388 at 1/100 dilution (5 µg/ml), followed by ab150077 AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in HUVEC cell line is observed. ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor®594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150077 AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution. 100% methanol fixation is recommended.
Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized 3T3-L1 (mouse embryonic fibroblast) starved with 0.4% serum for 24h, then cultured with 15% serum for 6h (Red) / Untreated control (Green) cells labeling Thrombospondin 1 with ab267388 at 1/50 (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HUVEC (human umbilical vein endothelial cell) cells labeling Thrombospondin 1 with ab267388 at 1/50 (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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