abcam

Product datasheet

Anti-TIMP1 antibody [EPR18352] ab211926





★★★★★ 10 Abreviews 30 References 13 Images

Overview

Product name Anti-TIMP1 antibody [EPR18352]

Description Rabbit monoclonal [EPR18352] to TIMP1

Host species Rabbit

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

Species reactivity Reacts with: Human

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human prostate cancer lysate; HeLa, SK-OV-3, HT-29, and U-87 MG whole cell lysates. IHC-

P: Human colon, pancreas, lung cancer, medullary thyroid carcinoma and prostate cancer tissues.

ICC/IF: SK-OV-3 and HT-29 cells.

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

Liquid **Form**

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, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR18352

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab211926 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-P	★★★★★(3)	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★ ☆☆ (1)	1/1000. Detects a band of approximately 26 kDa (predicted molecular weight: 23 kDa).
ICC/IF	★★★★ (3)	1/500.

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Function

Complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them by binding to their catalytic zinc cofactor. Also mediates erythropoiesis in vitro; but, unlike IL-3, it is species-specific, stimulating the growth and differentiation of only human and murine erythroid progenitors. Known to act on MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13 and MMP-16. Does not act on MMP-14.

Sequence similarities

Belongs to the protease inhibitor I35 (TIMP) family.

Contains 1 NTR domain.

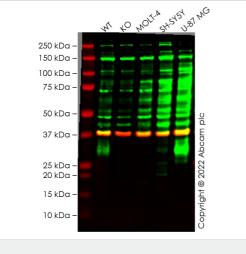
Post-translational

modifications

The activity of TIMP1 is dependent on the presence of disulfide bonds.

Cellular localization Secreted.

Images



Western blot - Anti-TIMP1 antibody [EPR18352] (ab211926)

All lanes : Anti-TIMP1 antibody [EPR18352] (ab211926) at 1/1000

dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: TIMP1 knockout HeLa cell lysate

Lane 3 : MOLT-4 cell lysate

Lane 4 : SH-SY5Y cell lysate

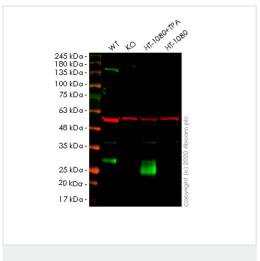
Lane 5 : U-87 MG cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 23 kDa Observed band size: 30 kDa

False colour image of Western blot: Anti-TIMP1 antibody [EPR18352] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab211926 was shown to bind specifically to TIMP1. A band was observed at 30 kDa in wild-type HeLa cell lysates with no signal observed at this size in TIMP1 knockout cell line ab264022 (knockout cell lysate ab260091). The identity of bands observed at higher molecular weights has not been determined. To generate this image, wild-type and TIMP1 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-TIMP1 antibody [EPR18352] (ab211926)

All lanes : Anti-TIMP1 antibody [EPR18352] (ab211926) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: TIMP1 knockout HeLa cell lysate

Lane 3: HT-1080 treated with 200ng/ml 12-O

Tetradecanoylphorbol-13-acetate (TPA) for 24 hours, cell lysate

Lane 4: Untreated HT-1080 cell lysate

Lysates/proteins at 20 µg per lane.

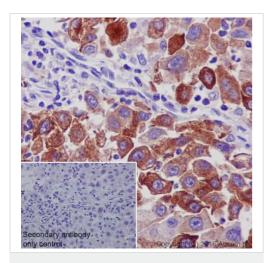
Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 23 kDa
Observed band size: 26 kDa

False colour image of Western blot: Anti-TIMP1 antibody [EPR18352] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab211926 was shown to bind specifically to TIMP1. A band was observed at 30 kDa in wild-type HeLa cell lysates with no signal observed at this size in TIMP1 knockout cell line ab264022 (knockout cell lysate ab260091). The identity of bands observed at higher molecular weights has not been determined. To generate this image, wild-type and TIMP1 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat

anti-Mouse IgG H&L 680RD at 1/20000 dilution.

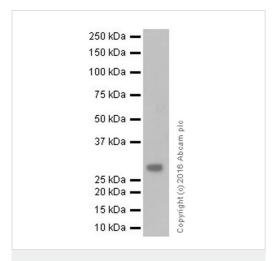


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TIMP1 antibody
[EPR18352] (ab211926)

Immunohistochemical analysis of paraffin-embedded human lung cancer tissue labeling TIMP1 with ab211926 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on human lung cancer 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.



Western blot - Anti-TIMP1 antibody [EPR18352] (ab211926)

Anti-TIMP1 antibody [EPR18352] (ab211926) at 1/1000 dilution + Human prostate cancer lysate at 10 μg

Secondary

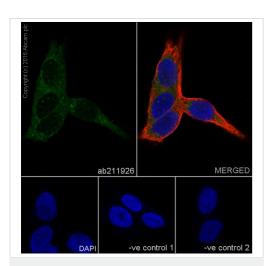
Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 23 kDa **Observed band size:** 26 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 16517973).



Immunocytochemistry/ Immunofluorescence - Anti-TIMP1 antibody [EPR18352] (ab211926)

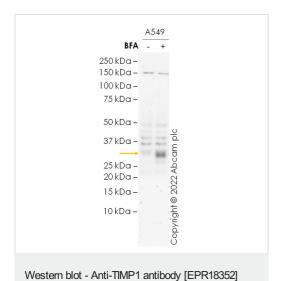
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SK-OV-3 (Human ovarian cancer cell line) cells labeling TIMP1 with ab211926 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on SK-OV-3 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (<u>ab150120</u>) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab211926 at 1/500 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution, followed by Goat Anti-Rabbit lgG (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.



(ab211926)

All lanes : Anti-TIMP1 antibody [EPR18352] (ab211926) at 1/1000 dilution

Lane 1: A549 Vehicle Control BFA (0 u/mL, 6 h) cell lysate Lane 2: A549 Treated BFA (5 u/mL, 6 h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 23 kDa **Observed band size:** 30 kDa

False colour image of Western blot: Anti-TIMP1 antibody

[EPR18352] staining at 1/1000 dilution, shown in black. In Western blot, ab211926 was shown to bind specifically to TIMP1. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween\$®\$ 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times before development with Optiblot (ECL reagent ab133456) and imaged with 20 minutes exposure time. Secondary antibodies used were HRP conjugated Goat anti-Rabbit (H+L) at 1/50000 dilution and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

2 250 kDa -250 kDa -150 kDa — 100 kDa — 75 kDa — 150 kDa -100 kDa -50 kDa -75 kDa -37 kDa -50 kDa -25 kDa -37 kDa -20 kDa -25 kDa -15 kDa -20 kDa -15 kDa -10 kDa -10 kDa -

Western blot - Anti-TIMP1 antibody [EPR18352] (ab211926)

All lanes : Anti-TIMP1 antibody [EPR18352] (ab211926) at 1/1000 dilution

Lane 1 : HT-29 (Human colorectal adenocarcinoma cell line) whole cell Ivsate

Lane 2: SK-OV-3 (Human ovarian cancer cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

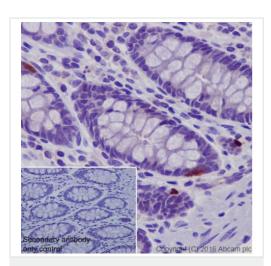
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 23 kDa Observed band size: 26 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Binding in these cell lines was extremely weak and requires optimization.

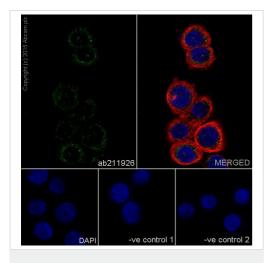


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TIMP1 antibody
[EPR18352] (ab211926)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling TIMP1 with ab211926 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on human colon neuroendocrine cell 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-TIMP1 antibody [EPR18352] (ab211926)

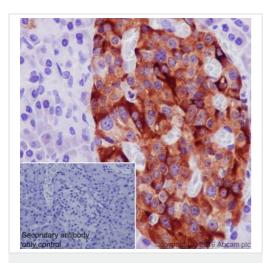
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HT-29 (Human colorectal adenocarcinoma cell line) cells labeling TIMP1 with ab211926 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HT-29 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab211926 at 1/500 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

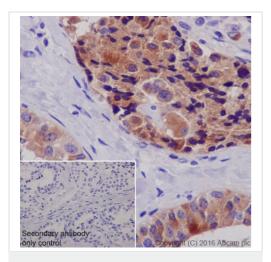


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TIMP1 antibody
[EPR18352] (ab211926)

Immunohistochemical analysis of paraffin-embedded human pancreas tissue labeling TIMP1 with ab211926 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on human islet is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.

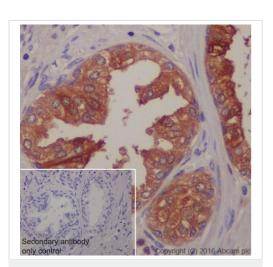


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TIMP1 antibody
[EPR18352] (ab211926)

Immunohistochemical analysis of paraffin-embedded human medullary thyroid carcinoma tissue labeling TIMP1 with ab211926 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on human medullary thyroid carcinoma 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.

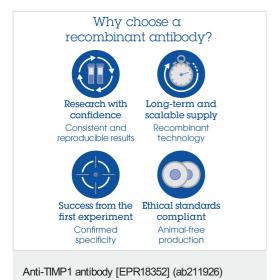


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TIMP1 antibody
[EPR18352] (ab211926)

Immunohistochemical analysis of paraffin-embedded human prostate cancer tissue labeling TIMP1 with ab211926 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic staining on human prostate cancer 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.



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