# abcam

### Product datasheet

# Anti-TGF beta 1 antibody [EPR21143] - BSA and Azide free ab229856





RabMAb

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#### Overview

Product name Anti-TGF beta 1 antibody [EPR21143] - BSA and Azide free

**Description** Rabbit monoclonal [EPR21143] to TGF beta 1 - BSA and Azide free

Host species Rabbit

**Specificity** For testing samples with low expression level of TGF beta 1, we recommend <u>ab179695</u> which

could give stronger signal. Loading larger amount of lysate or lower antibody dilution would also

help.

**Tested applications** Suitable for: IHC-P, WB

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control IHC-P: Human thrombocytosis tissue; human bone; Mouse and rat spleen tissues. WB: NIH/3T3,

L-929, HeLa, A549, HL-60, RAW 264.7, Wild-type A549, K562 and SH-SY5Y whole cell lysates;

Mouse and Rat spleen lysate; Mouse heart tissue lysate; C6 cell lysa

**General notes** ab229856 is the carrier-free version of <u>ab215715</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

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- 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**.

#### **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 EPR21143

**Isotype** IgG

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab229856 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		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 44 kDa.

## **Target**

**Function** Multifunctional protein that controls proliferation, differentiation and other functions in many cell

types. Many cells synthesize TGFB1 and have specific receptors for it. It positively and negatively regulates many other growth factors. It plays an important role in bone remodeling as it is a potent stimulator of osteoblastic bone formation, causing chemotaxis, proliferation and differentiation in

committed osteoblasts.

Tissue specificity Highly expressed in bone. Abundantly expressed in articular cartilage and chondrocytes and is

increased in osteoarthritis (OA). Co-localizes with ASPN in chondrocytes within OA lesions of

articular cartilage.

Involvement in disease Defects in TGFB1 are the cause of Camurati-Engelmann disease (CE) [MIM:131300]; also

known as progressive diaphyseal dysplasia 1 (DPD1). CE is an autosomal dominant disorder characterized by hyperostosis and sclerosis of the diaphyses of long bones. The disease typically presents in early childhood with pain, muscular weakness and waddling gait, and in some cases

other features such as exophthalmos, facial paralysis, hearing difficulties and loss of vision.

**Sequence similarities**Belongs to the TGF-beta family.

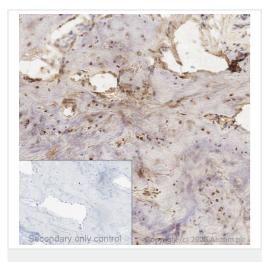
Post-translational Glycosylated.

modifications The precursor is cleaved into mature TGF-beta-1 and LAP, which remains non-covalently linked to

mature TGF-beta-1 rendering it inactive.

**Cellular localization** Secreted > extracellular space > extracellular matrix.

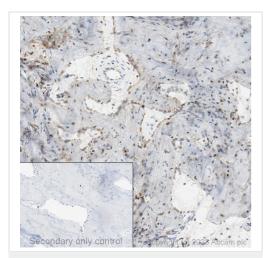
#### **Images**



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TGF beta 1 antibody
[EPR21143] - BSA and Azide free (ab229856)

Immunohistochemical analysis of formalin-fixed paraffin-embedded human bone labelling TGF beta 1 with <u>ab215715</u> at a concentration of 3µg/ml. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument with a Bond<sup>™</sup> Polymer Refine Detection kit. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20 mins. <u>ab215715</u> anti-TGF beta 1 antibody [EPR21143] was incubated for 15mins at room temperature. Sections were counterstained with Hematoxylin. Image inset shows absence of staining in secondary antibody only control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab215715</u>).

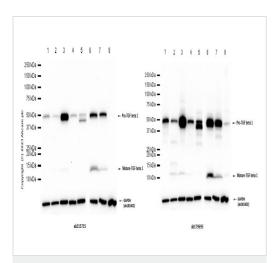


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TGF beta 1 antibody

[EPR21143] - BSA and Azide free (ab229856)

Immunohistochemical analysis of formalin-fixed paraffin-embedded human bone labelling TGF beta 1 with <a href="mailto:ab215715">ab215715</a> at a dilution of 4µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5). <a href="mailto:ab215715">ab215715</a> anti TGF beta 1 antibody was incubated at 37°C for 16min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab215715**).



Western blot - Anti-TGF beta 1 antibody [EPR21143] - BSA and Azide free (ab229856)

**All lanes :** Anti-TGF beta 1 antibody [EPR21143] (ab215715) at 1/1000 dilution

Lane 1 : A549 (Human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2 : HL-60 (Human acute promyelocytic leukemia promyeloblast) whole cell lysate at 20 µg

Lane 3: K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate at 20 µg

**Lane 4 :** NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at  $20 \ \mu g$ 

Lane 5: C6 (Rat glial tumor glial cell) whole cell lysate at 20 µg

Lane 6 : Mouse spleen tissue lysateLane 7 : Rat spleen tissue lysateLane 8 : Mouse heart tissue lysate

#### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 44 kDa

Observed band size: 12,44 kDa

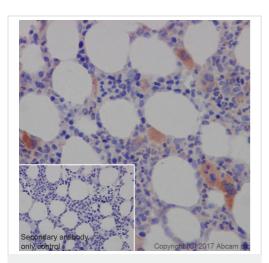
Exposure time: 20 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215715).

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

ab181602 was used as a GAPDH loading control.

For testing samples with low expression level of TGF beta 1, we recommend <u>ab179695</u> which could give stronger signal. Loading larger amount of lysate or lower antibody dilution would also help.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TGF beta 1 antibody

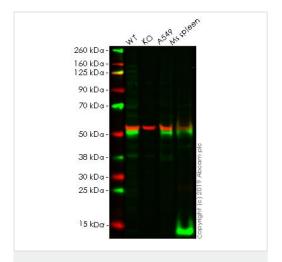
[EPR21143] - BSA and Azide free (ab229856)

Immunohistochemical analysis of paraffin-embedded human thrombocytosis tissue labeling TGF beta 1 with <u>ab215715</u> at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) ready to use. Cytoplasmic staining of megakaryocytes in human thrombocytosis (PMID: 25305163).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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 (ab215715).

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



Western blot - Anti-TGF beta 1 antibody [EPR21143] - BSA and Azide free (ab229856)

**All lanes :** Anti-TGF beta 1 antibody [EPR21143] (**ab215715**) at 1/1000 dilution

Lane 1: Wild-type HeLa whole cell lysate

Lane 2: TGFB1 knockout HeLa whole cell lysate

Lane 3: A549 whole cell lysate

Lane 4: Mouse spleen tissue lysate

Lysates/proteins at 20 µg per lane.

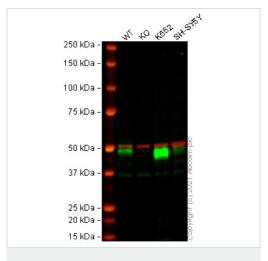
Predicted band size: 44 kDa Observed band size: 13,44 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab215715</u> observed at 13 and 44 kDa. Red - loading control, <u>ab7291</u>, observed at 50 kDa.

ab215715 was shown to specifically react with in wild-type HeLa cells as signal was lost in TGFB1 knockout cells. Wild-type and TGFB1 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% NF Milk. Ab215715 and ab7291 (Mouse anti-tubulin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were

developed with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab215715).



Western blot - Anti-TGF beta 1 antibody
[EPR21143] - BSA and Azide free (ab229856)

**All lanes :** Anti-TGF beta 1 antibody [EPR21143] (ab215715) at 1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: TGFB1 knockout A549 cell lysate

Lane 3: K562 cell lysate

Lane 4: SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

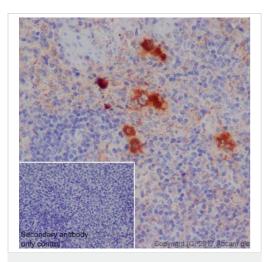
Performed under reducing conditions.

Predicted band size: 44 kDa Observed band size: 48 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab215715).

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab215715</u> observed at 48 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab215715 was shown to react with TGF beta 1 in wild-type A549 cells in Western blot with loss of signal observed in TGFB1 knockout cell line ab269509 (TGFB1 knockout cell lysate ab269671). Wild-type A549 and TGFB1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab215715 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TGF beta 1 antibody

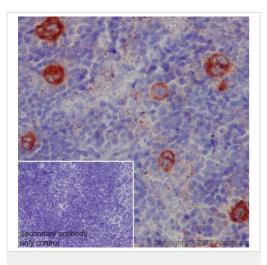
[EPR21143] - BSA and Azide free (ab229856)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling TGF beta 1 with <u>ab215715</u> at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) ready to use. Positive staining of megakaryocytes and platelets in rat spleen (PMID: 25305163).

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 (ab215715).

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TGF beta 1 antibody

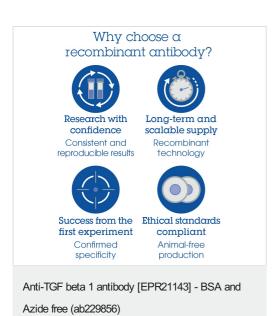
[EPR21143] - BSA and Azide free (ab229856)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling TGF beta 1 with <u>ab215715</u> at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) ready to use. Positive staining of megakaryocytes and platelets in mouse spleen (PMID: 25305163).

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 (ab215715).

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



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