abcam

Product datasheet

Anti-SPARC antibody [EPR25122-122] - BSA and Azide free ab290647

Recombinant RabMAb

13 Images

Overview

Product name Anti-SPARC antibody [EPR25122-122] - BSA and Azide free

Description Rabbit monoclonal [EPR25122-122] to SPARC - BSA and Azide free

Host species Rabbit

Specificity This antibody does not react with Rat species for IHC application.

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

Species reactivity Reacts with: Mouse. Rat

Does not react with: Human

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

Positive control WB: Mouse brain, heart, placenta tissue lysate. Rat brain, heart, placenta tissue lysates. C2C12,

> Neuro-2a, bEnd.3 and C6 whole cell lysate, untreated mouse and rat brain tissue lysate, mouse and rat brain tissue lysate treated with Protein Deglycosylation MIX II. Flow Cyt (intra): rat and mouse primary neuron cells. IP: Neuro-2a whole cell lysate, rat brain tissue. IHC-P: mouse hepatocellular, kidney, cerebrum tissues. ICC/IF: Permeabilized bEnd.3 and C6 cells.

General notes ab290647 is a carrier free version of ab290636.

> Our carrier-free 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 cellbased 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 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

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C.

Storage buffer pH: 7.2

Constituent: 100% PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR25122-122

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab290647 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. Predicted molecular weight: 35 kDa.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

Target

Function Appears to regulate cell growth through interactions with the extracellular matrix and cytokines.

Binds calcium and copper, several types of collagen, albumin, thrombospondin, PDGF and cell membranes. There are two calcium binding sites; an acidic domain that binds 5 to 8 Ca(2+) with

a low affinity and an EF-hand loop that binds a Ca(2+) ion with a high affinity.

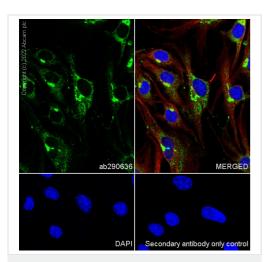
Sequence similarities Belongs to the SPARC family.

Contains 1 EF-hand domain.
Contains 1 follistatin-like domain.
Contains 1 Kazal-like domain.

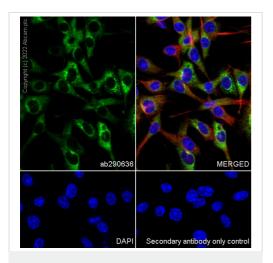
Developmental stage Expressed at high levels in tissues undergoing morphogenesis, remodeling and wound repair.

Cellular localization Secreted > extracellular space > extracellular matrix > basement membrane. In or around the

Images



Immunocytochemistry/ Immunofluorescence - Anti-SPARC antibody [EPR25122-122] - BSA and Azide free (ab290647)



Immunocytochemistry/ Immunofluorescence - Anti-SPARC antibody [EPR25122-122] - BSA and Azide free (ab290647)

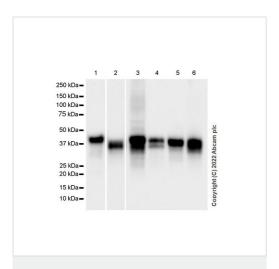
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized bEnd.3 (mouse brain endothelioma cell line) cells labelling SPARC with primary antibody anti-SPARC (ab290636) at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor 488) (ab150081) secondary antibody at 1/1000 dilution (2.0 μ g/mL). Confocal image showing cytoplasmic staining in bEnd.3 cells. Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor 594) (ab195889) was used to counterstain tubulin at 1/200 dilution (2.5 μ g/mL). The nuclear counter stain is DAPI (blue).

The secondary antibody only control is : Secondary antibody is $\underline{ab150081} \text{ Goat Anti-Rabbit IgG H\&L (Alexa Fluor}^{\$}488) \text{ at 1/1000 dilution (2.0 $\mu\text{g/mL}$)}.$

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized C6 (rat glial tumour cell line) cells labelling SPARC with primary antibody anti-SPARC ($\underline{ab290636}$) at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor 488) ($\underline{ab150081}$) secondary antibody at 1/1000 dilution (2.0 μ g/mL). Confocal image showing cytoplasmic staining in C6 cells. Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor 594) ($\underline{ab195889}$) was used to counterstain tubulin at 1/200 dilution (2.5 μ g/mL). The nuclear counter stain is DAPI (blue). The secondary antibody only control is : Secondary antibody is $\underline{ab150081}$ Goat Anti-Rabbit IgG H&L (Alexa Fluor 488) at 1/1000 dilution (2.0 μ g/mL).

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.



Western blot - Anti-SPARC antibody [EPR25122-122] - BSA and Azide free (ab290647) **All lanes :** Anti-SPARC antibody [EPR25122-122] (ab290636) at 1/1000 dilution

Lane 1: Mouse brain tissue lysate

Lane 2: Mouse heart tissue lysate

Lane 3: Mouse placenta tissue lysate

Lane 4: Rat brain tissue lysate

Lane 5: Rat heart tissue lysate

Lane 6: Rat placenta tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 35 kDa **Observed band size:** 40, 37 kDa

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

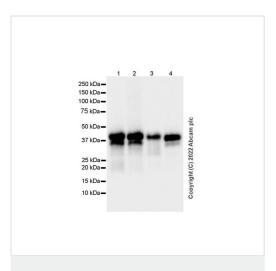
Blocking and diluting buffer and concentration was 5% NFDM/TBST.

The expression profile/molecular weight observed is consistent with that described in the literature (PMID: 29449802; PMID: 9008236). The additional band is expected to be a SPARC cleavage product (PMID: 9008236).

Exposure time:

Lane 2: 5.5 seconds

Lane 1, 3-6: 3.25 seconds



Western blot - Anti-SPARC antibody [EPR25122-122] - BSA and Azide free (ab290647) **All lanes :** Anti-SPARC antibody [EPR25122-122] (<u>ab290636</u>) at 1/1000 dilution

Lane 1: C2C12 (mouse myoblast) whole cell lysate

Lane 2: bEnd.3 (mouse brain endothelioma) whole cell lysate

Lane 3: Neuro-2a (mouse neuroblastoma) whole cell lysate

Lane 4: C6 (rat glioma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

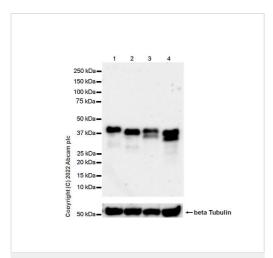
Predicted band size: 35 kDa **Observed band size:** 40, 37 kDa

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

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

Exposure time: 3.25 seconds.

The expression profile/molecular weight observed is consistent with that described in the literature (PMID: 29449802; PMID: 9008236). The additional band is expected to be a SPARC cleavage product (PMID: 9008236).



Western blot - Anti-SPARC antibody [EPR25122-122] - BSA and Azide free (ab290647) **All lanes :** Anti-SPARC antibody [EPR25122-122] (<u>ab290636</u>) at 1/1000 dilution

Lane 1: Untreated mouse brain tissue lysate

Lane 2: Mouse brain tissue lysate treated with Protein

Deglycosylation MIX II

Lane 3: Untreated rat brain tissue lysate

Lane 4: Rat brain tissue lysate treated with Protein

Deglycosylation MIX II

Lysates/proteins at 15 µg per lane.

Secondary

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

Predicted band size: 35 kDa **Observed band size:** 40, 37 kDa

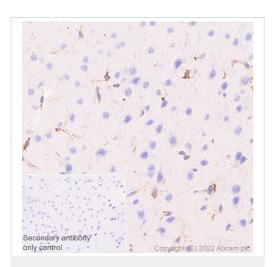
Exposure time: 103 seconds

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

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

The expression profile/molecular weight observed is consistent with that described in the literature (PMID: 29449802; PMID: 9008236). SPARC is a glycosylated protein and can be deglycosylated by Protein Deglycosylation MIX II.

The additional band is expected to be a SPARC cleavage product (PMID: 9008236).



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

[EPR25122-122] - BSA and Azide free (ab290647)

Secondary antibody only control

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

[EPR25122-122] - BSA and Azide free (ab290647)

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labelling SPARC with <u>ab290636</u> at 1/15000 (0.041 µg/ml) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on microglia of mouse cerebrum. The section was incubated with <u>ab290636</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection) .

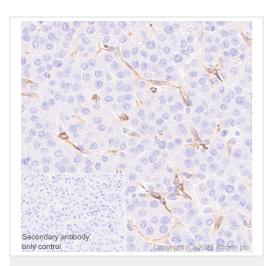
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labelling SPARC with <u>ab290636</u> at 1/15000 (0.041 µg/ml) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on glomerulus and endothelium of mouse kidney. The section was incubated with <u>ab290636</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



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

[EPR25122-122] - BSA and Azide free (ab290647)

Flow Cytometry (Intracellular) - Anti-SPARC antibody [EPR25122-122] - BSA and Azide free (ab290647)

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

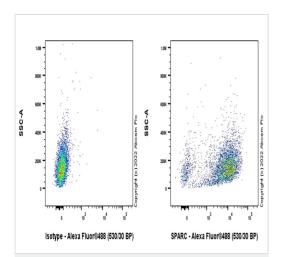
Immunohistochemical analysis of paraffin-embedded mouse hepatocellular tissue labelling SPARC with <u>ab290636</u> at 1/15000 (0.041 µg/ml) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on interstitial cells of mouse hepatocellular carcinoma. The section was incubated with <u>ab290636</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection) .

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

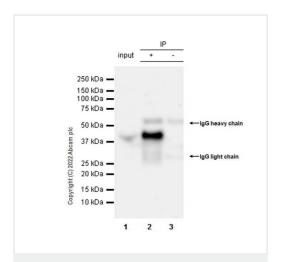
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Mouse primary neuron cell cells labelling SPARC with <u>ab290636</u> at 1/600 dilution (0.1µg) (Right) compared with a Rabbit monoclonal IgG (<u>ab172730</u>) (Left) isotype control 0. A Goat Anti-Rabbit IgG (Alexa Fluor[®] 488, <u>ab150081</u>) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-SPARC antibody [EPR25122-122] - BSA and Azide free (ab290647)

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Rat primary neuron cells labelling SPARC with <u>ab290636</u> at 1/600 dilution (0.1 μ g) (Right) compared with a Rabbit monoclonal lgG (<u>ab172730</u>) (Left). A Goat Anti-Rabbit lgG (Alexa Fluor® 488, <u>ab150081</u>) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-SPARC antibody
[EPR25122-122] - BSA and Azide free (ab290647)

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

SPARC was immunoprecipitated from Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate with <u>ab290636</u> at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab290636</u> at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

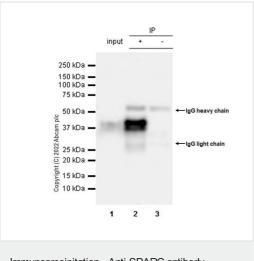
Lane 1: Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate 10 µg

Lane 2: ab290636 IP in Neuro-2a whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab290636</u> in Neuro-2a whole cell lysate

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

Exposure time: 10 seconds



Immunoprecipitation - Anti-SPARC antibody
[EPR25122-122] - BSA and Azide free (ab290647)

This data was developed using <u>ab290636</u>, the same antibody clone in a different buffer formulation.

SPARC was immunoprecipitated from rat brain tissue lysate with ab290636 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab290636 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: Rat brain tissue lysate 10 µg

Lane 2: ab290636 IP in Rat brain tissue lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab290636</u> in rat brain tissue lysate

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

Exposure time: 10 seconds



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