abcam

Product datasheet

Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free ab220213

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free
Description	Rabbit monoclonal [EPR14639(2)] to S100A4 - BSA and Azide free
Host species	Rabbit
Specificity	Based on sequence homologies, the antibody may cross-react with other proteins of the same family (S100A1-12). We did not perform any experiments to confirm this. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, A549, and A375 cell lysates and human fetal spleen tissue lysates. IHC-P: Human cervix carcinoma, lung carcinoma and gastric carcinoma tissues. ICC/IF: Jurkat cells. Flow Cyt (intra): Jurkat cells.
General notes	ab220213 is the carrier-free version of **ab197896**. 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 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® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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	EPR14639(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab220213 in the following tested applications.

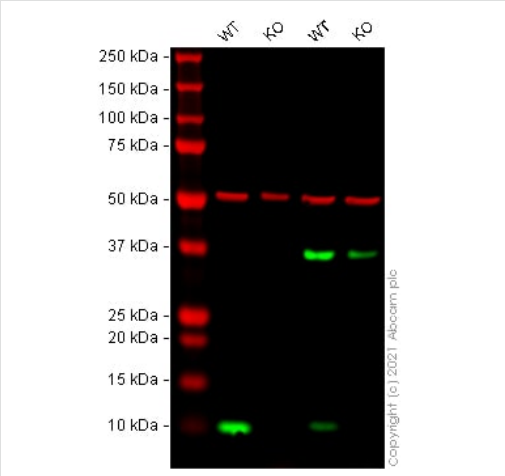
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 12 kDa (predicted molecular weight: 12 kDa).
IHC-P	★★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. We do not guarantee IHC-P for mouse and rat.

Target

Tissue specificity	Ubiquitously expressed.
Sequence similarities	Belongs to the S-100 family. Contains 2 EF-hand domains.

Images



Western blot - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

All lanes : Anti-S100A4 antibody [EPR14639(2)] (**ab197896**) at 1/1000 dilution

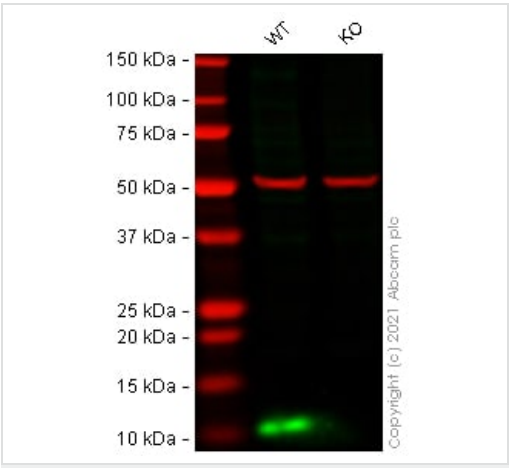
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : S100A4 knockout HeLa cell lysate
Lane 3 : Wild-type A549 cell lysate
Lane 4 : S100A4 knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 12 kDa
Observed band size: 11 kDa

False colour image of Western blot: Anti-S100A4 antibody [EPR14639(2)] staining at 1/1000 dilution, shown in green; loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) staining at 1/20000 dilution, shown in red. In Western blot, **ab197896** was shown to bind specifically to S100A4. A band was observed at 11 kDa in wild-type HeLa and A549 cell lysates with no signal observed at this size in S100A4 knockout HeLa cell line **ab265709** (knockout cell lysate **ab257046**) and S100A4 knockout A549 cell line **ab261865** (knockout cell lysate **ab261674**). To generate this image, wild-type and S100A4 knockout HeLa and S100A4 knockout A549 cell lysates were analysed. 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 then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

All lanes : Anti-S100A4 antibody [EPR14639(2)] (**ab197896**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : S100A4 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

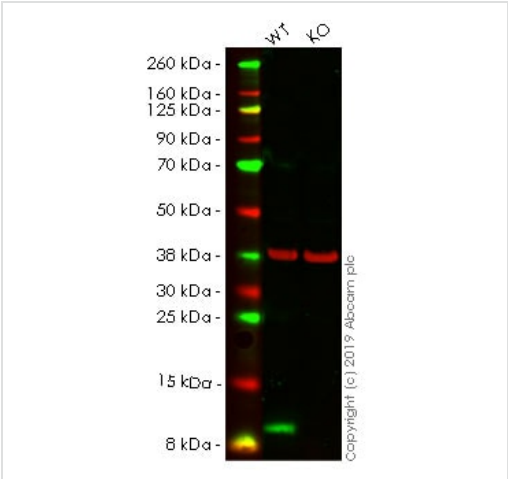
Performed under reducing conditions.

Predicted band size: 12 kDa
Observed band size: 11 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab197896**).

Lanes 1 - 2: Merged signal (red and green). Green - **ab197896** observed at 11 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab197896 was shown to react with S100A4 in wild-type HeLa cells in Western blot with loss of signal observed in S100A4 knockout cell line **ab265709** (S100A4 knockout cell lysate **ab257046**). Wild-type HeLa and S100A4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab197896** 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.



Western blot - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

All lanes : Anti-S100A4 antibody [EPR14639(2)] (**ab197896**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : S100A4 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

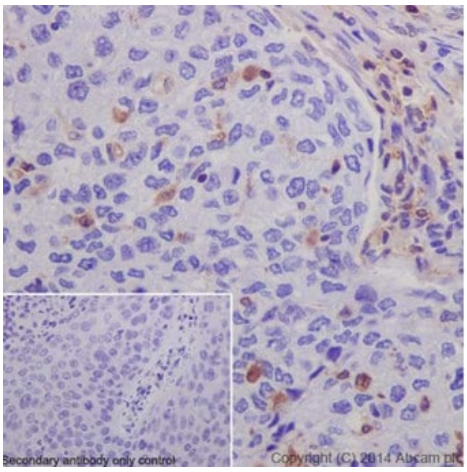
Performed under reducing conditions.

Predicted band size: 12 kDa
Observed band size: 12 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab197896**).

Lanes 1 - 2: Merged signal (red and green). Green - **ab197896** observed at 12 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab197896 Recombinant Anti-S100A4 antibody [EPR14639(2)] was shown to specifically react with S100A4 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab261758** (knockout cell lysate **ab257045**) was used. Wild-type and S100A4 knockout samples were subjected to SDS-PAGE. **ab197896** and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (**ab52866**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



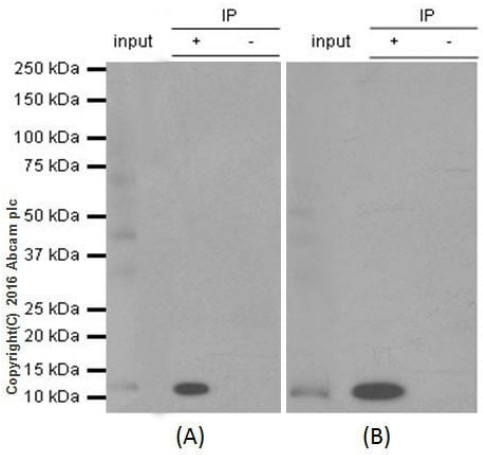
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling S100A4 using **ab197896** at 1/2000 dilution. Goat Anti-Rabbit IgG H&L (HRP) **ab97051** was used ay 1/500 dilution as a secondary antibody and cells were counterstained with Hematoxylin.

Inset image: negative control obtained using PBS instead of **ab197896** and secondary antibody only.
Note: Nuclear and cytoplasm staining on cervix carcinoma tissue was observed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab197896**).

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



Immunoprecipitation - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

ab197896 at 1/40 immunoprecipitating S100A4 in A549 whole cell lysate observed at 12 KDa.

Lane 1 (input): A549 whole cell lysate 10µg

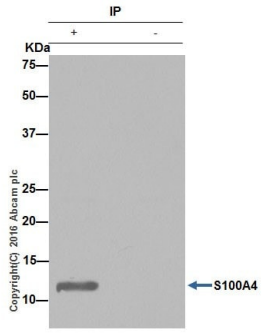
Lane 2 (+): **ab197896** + A549 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab197896** in A549 whole cell lysate

For western blotting, Panel A: **ab197896**, 1:1000; Panel B: **ab124805**, 1:1000 and anti-rabbit IgG (HRP), specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking/Diluting 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 (**ab197896**).



Immunoprecipitation - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

ab197896 at 1/40 immunoprecipitating S100A4 in A549 whole cell lysate observed at 12 KDa.

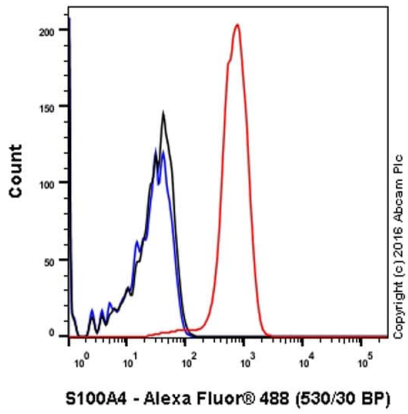
Lane 1 (+): **ab197896** + A549 whole cell lysate.

Lane 2 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab197896** in A549 whole cell lysate

For western blotting, **ab197896** at 1/1000 and anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG (1/1500).

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

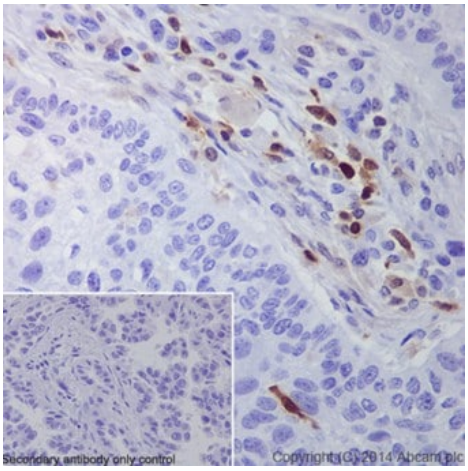
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab197896**).



Flow Cytometry (Intracellular) - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

Intracellular Flow Cytometry analysis of Jurkat cells labelling S100A4 with **ab197896** at 1/250 (red). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab197896**).



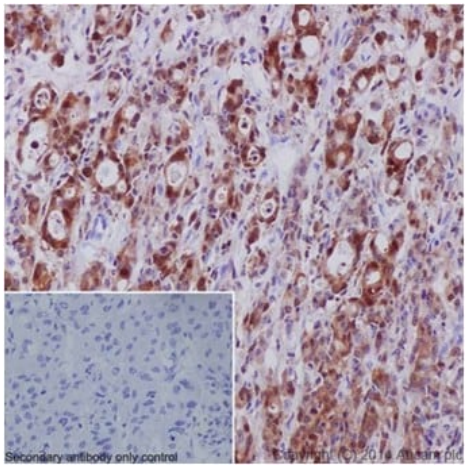
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

Immunohistochemical analysis of paraffin-embedded Human lung carcinoma tissue labeling S100A4 using **ab197896** at 1/2000 dilution. Goat Anti-Rabbit IgG H&L (HRP) **ab97051** was used as a secondary antibody at a dilution of 1/500 and cells were counterstained with Hematoxylin.

Inset image: negative control obtained using PBS instead of **ab197896** and secondary antibody only.
Note: Nuclear and weakly staining on lung carcinoma tissue was observed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab197896**).

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



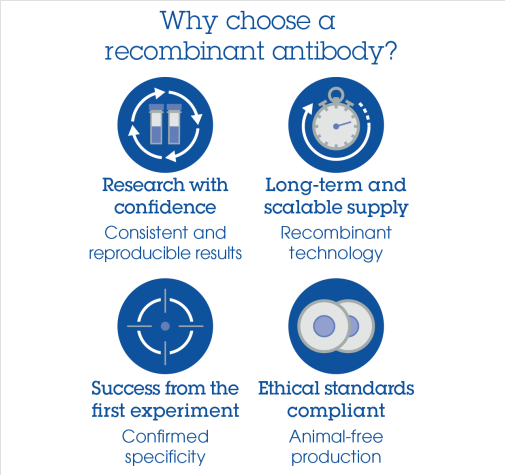
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

Immunohistochemical analysis of paraffin-embedded Human gastric carcinoma tissue labeling S100A4 using **ab197896** at 1/2000 dilution. Goat Anti-Rabbit IgG H&L (HRP) **ab97051** was used as a secondary antibody at 1/500 dilution. Cells were counterstained with Hematoxylin.

Inset image: negative control obtained using PBS instead of **ab197896** and secondary antibody only.
Note: Cytoplasm and nuclear staining on human gastric carcinoma tissue was observed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab197896**).

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



Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

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