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. Some optimisation may be required for detection of the target protein due to low levels of endogenous expression in some samples. Please see images below for suitable positive controls.
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.

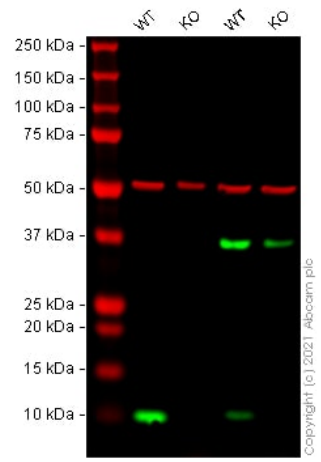
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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

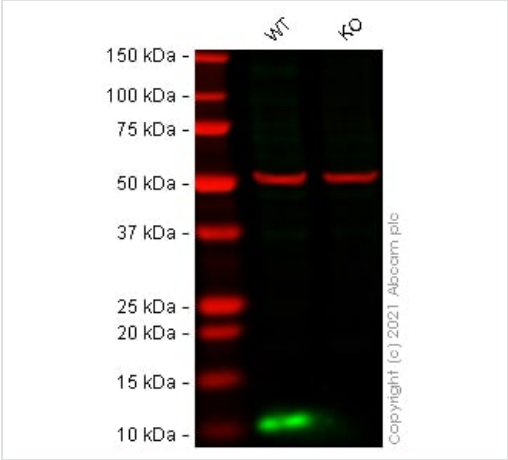
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : S100A4 knockout HeLa cell lysate
Lane 3 : Wild-type A549 cell lysate
Lane 4 : S100A4 knockout A549 cell lysate

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.

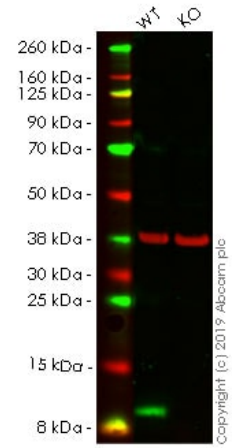
Performed under reducing conditions.

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.

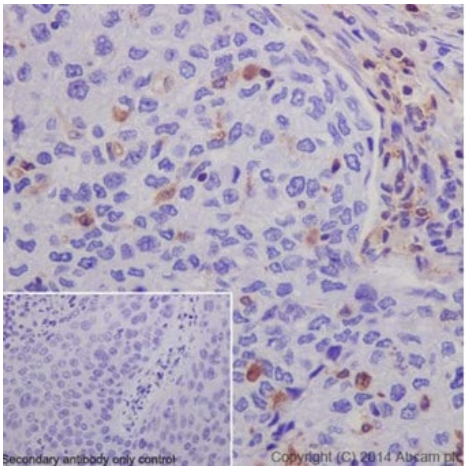
Performed under reducing conditions.

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.



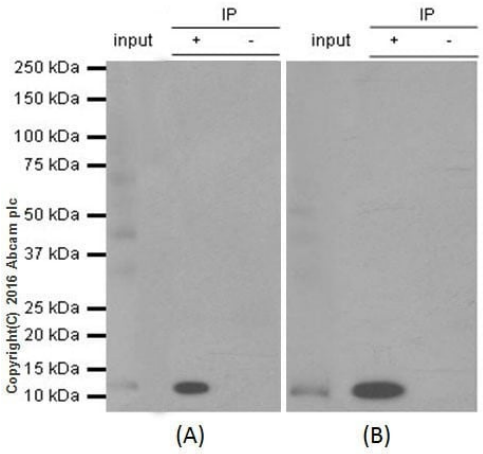
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

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

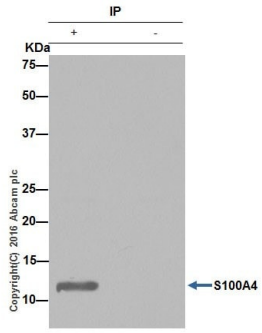
Lane 2 (+): **ab197896** + A549 whole cell lysate.

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.

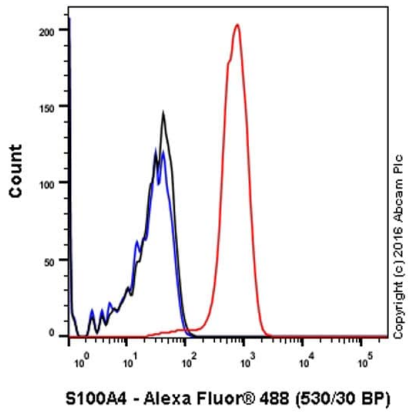
Lane 1 (+): **ab197896** + A549 whole cell lysate.

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.

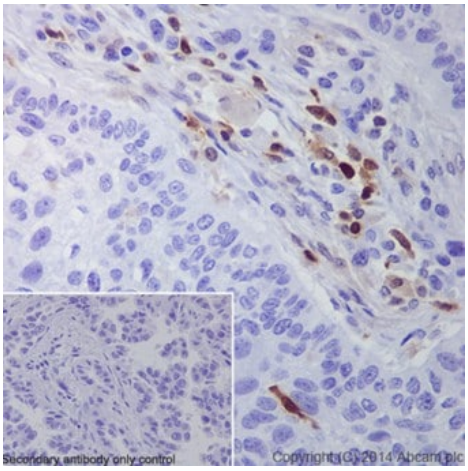
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab197896**).



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**).



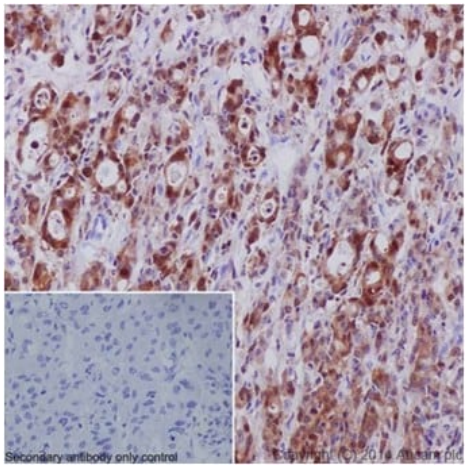
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

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.



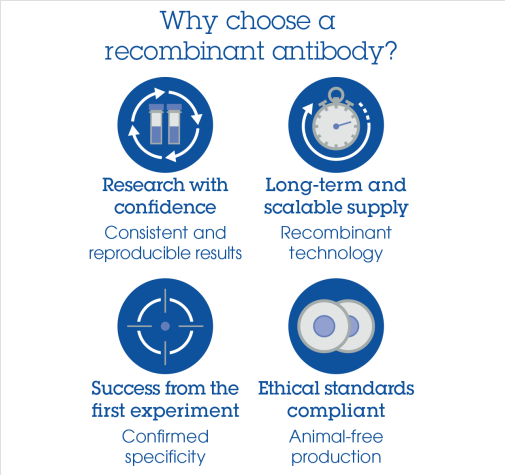
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S100A4 antibody [EPR14639(2)] - BSA and Azide free (ab220213)

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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