abcam

Product datasheet

Anti-S100A4 antibody [EPR14639(2)] ab197896

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-S100A4 antibody [EPR14639(2)]
Description	Rabbit monoclonal [EPR14639(2)] to S100A4
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), IHC-P, WB, ICC/IF, IP
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: A549, A375, HeLa, NIH/3T3, Raw264.7 cell lysates. Human fetal spleen and colon tissue lysates. Mouse spleen and bone marrow tissues lysates. IHC-P: Human cervix carcinoma, lung carcinoma and gastric carcinoma tissues, rat spleen tissue. ICC/IF: HeLa cells. Flow Cyt (intra): Jurkat 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

Form	Liquid
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: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR14639(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab197896 in the following tested applications.

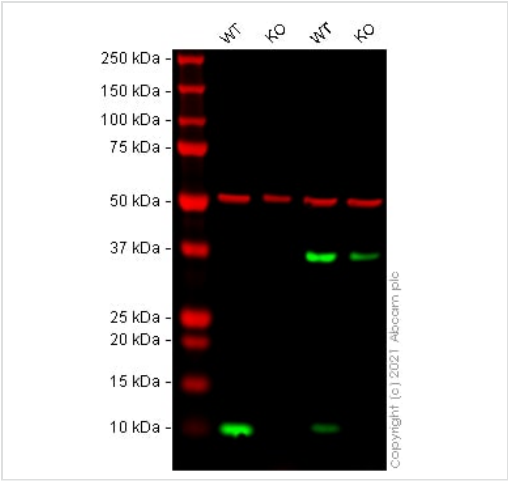
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/250.
IHC-P	★★★★☆ (3)	1/2000. 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.
WB		1/1000. Detects a band of approximately 12 kDa (predicted molecular weight: 12 kDa).
ICC/IF	★★★★☆ (2)	1/250.
IP		1/40.

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)] (ab197896)

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

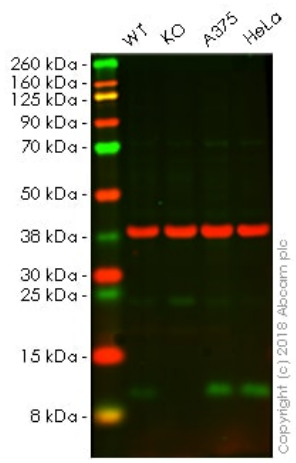
Lysates/proteins at 20 µg per lane.

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)] (ab197896)

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

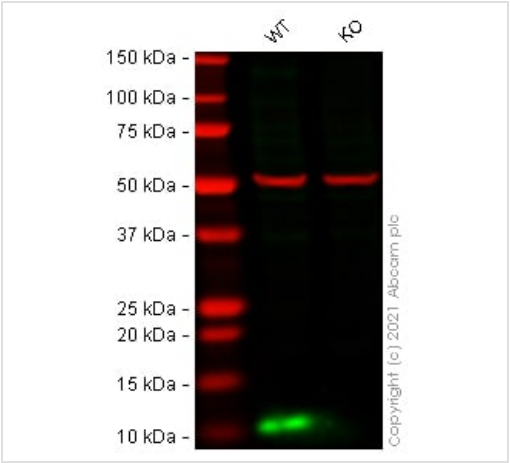
Lane 1 : Wild-type A549 whole cell lysate
Lane 2 : S100A4 knockout A549 whole cell lysate
Lane 3 : A375 whole cell lysate
Lane 4 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Lanes 1 - 4: Merged signal (red and green). Green - ab197896 observed at 12 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab197896 was shown to recognize S100A4 in wild-type A549 cells as signal was lost at the expected MW in S100A4 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and S100A4 knockout samples were subjected to SDS-PAGE. Ab197896 and **ab8245** (Mouse anti GAPDH 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® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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

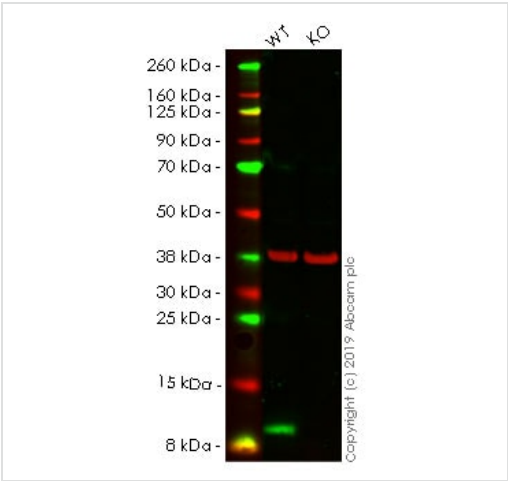
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

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)] (ab197896)

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

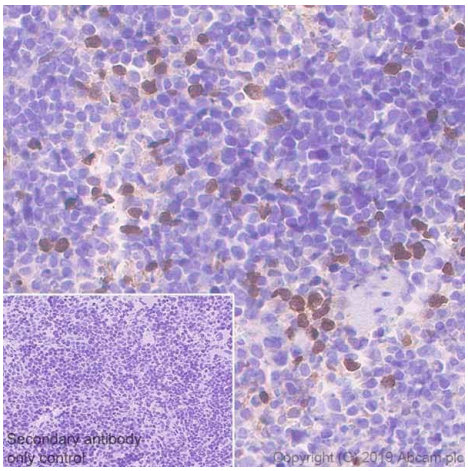
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

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

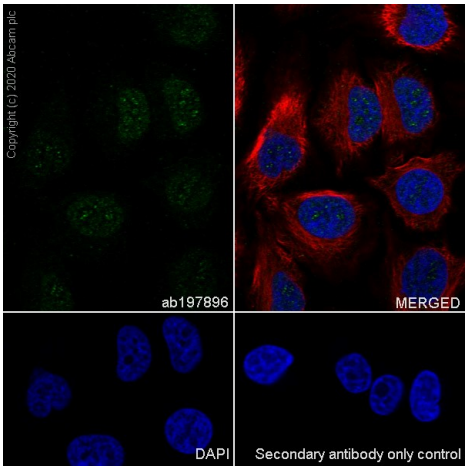
ab197896 Recombinant Anti-S100A4 antibody [EPR14639(2)] was shown to specifically react with S100A4 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab261758** (knockout cell lysate **ab257045**) was used. Wild-type and S100A4 knockout samples were subjected to SDS-PAGE. 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)] (ab197896)

Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling S100A4 with ab197896 at 1/2000 dilution (0.376 µg/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on lymphocytes of rat spleen.The section was incubated with ab197896 for 30 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

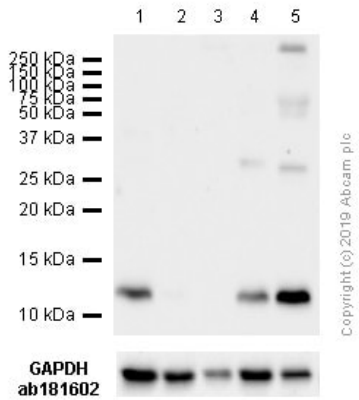
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).



Immunocytochemistry/ Immunofluorescence - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling S100A4 with ab197896 at 1/200 (10 µg/mL). Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% TritonX-100. **ab150077**, AlexaFluor®488 Goat anti-Rabbit at 1/1000 (2 µg/mL) was used as the secondary antibody. Cells were counterstained with **ab195889**, Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 µg/mL). Nuclear counter stain was DAPI (blue).

Confocal image showing positive staining in HeLa cell line.



Western blot - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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

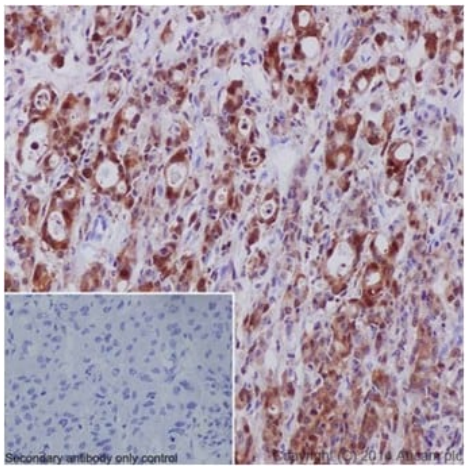
Lanes 1 & 5 : A549 whole cell lysate
Lane 2 : Human liver lysates
Lane 3 : Human lung lysates
Lane 4 : Human colon lysates

Lysates/proteins at 20 µg per lane.

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

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

Exposure time: 26 seconds

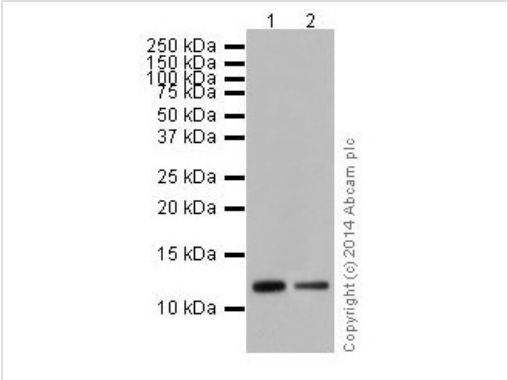


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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

Lane 1 : A375 (human malignant melanoma) whole cell lysate
Lane 2 : Human fetal spleen lysate

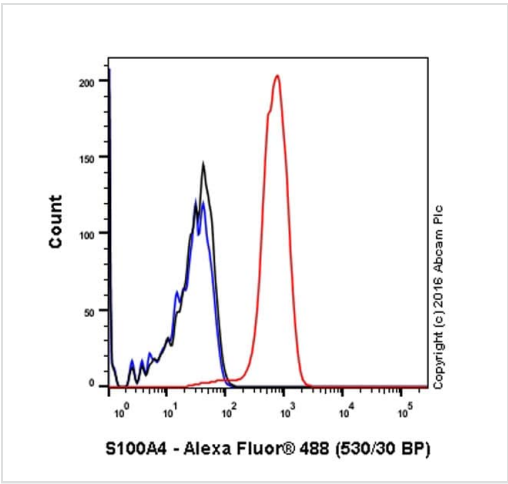
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 12 kDa

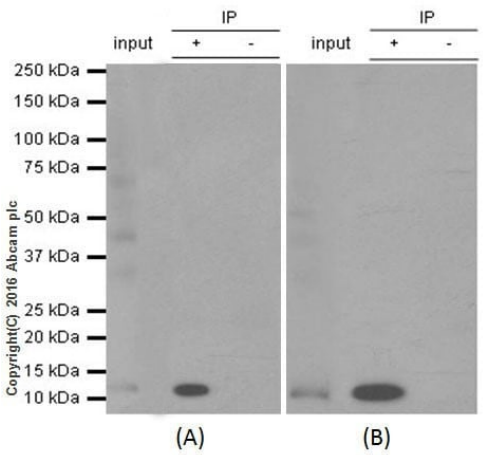
Exposure time: 3 minutes

Blocking/dilution buffer: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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 Fluorr® 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.



Immunoprecipitation - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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

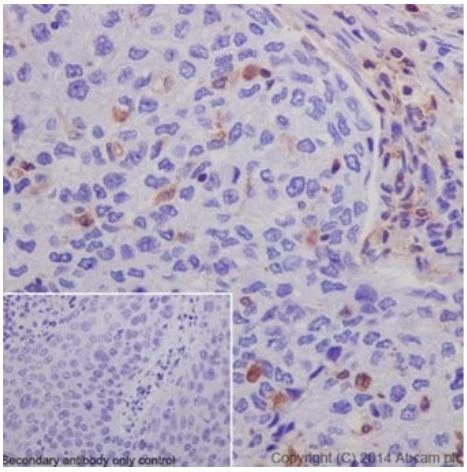
Lane 1 (input): A549 whole cell lysate 10µg

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.

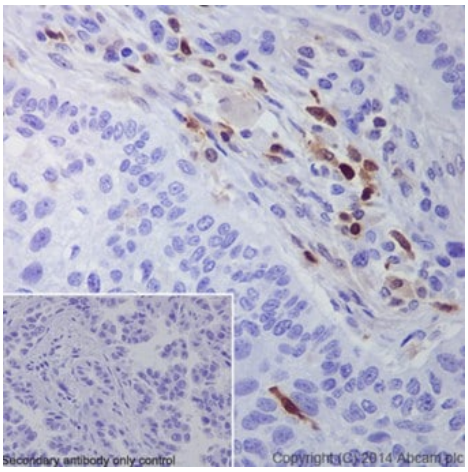


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

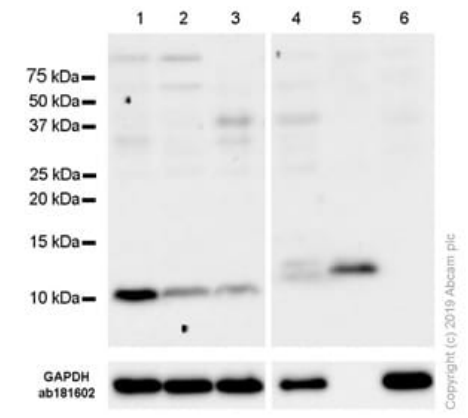


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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

Lane 1 : A549 (Human lung carcinoma epithelial cell) whole cell lysates
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 3 : Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates
Lane 4 : Mouse spleen tissue lysates
Lane 5 : Mouse bone marrow tissue lysates
Lane 6 : Mouse heart tissue lysates

Lysates/proteins at 20 µg per lane.

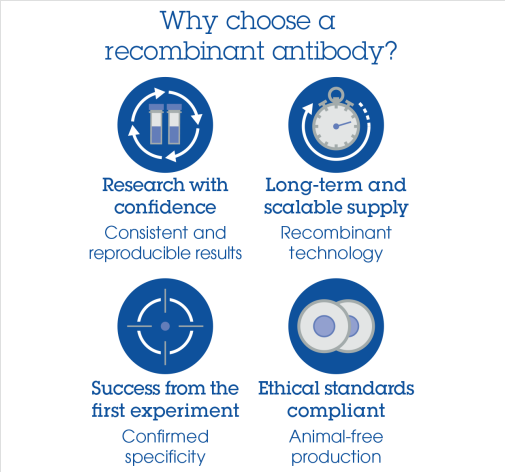
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Exposure time: 44 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

Some optimisation may be required for detection of the target protein due to low levels of endogenous expression in some samples.



Anti-S100A4 antibody [EPR14639(2)] (ab197896)

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