# abcam

## Product datasheet

## Anti-S100A4 antibody [EPR2761(2)] ab124805





★★★★★ 8 Abreviews 50 References 14 Images

#### Overview

**Product name** Anti-S100A4 antibody [EPR2761(2)]

**Description** Rabbit monoclonal [EPR2761(2)] to S100A4

**Host species** Rabbit

Specificity 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: ICC/IF, Flow Cyt (Intra), WB, IP, IHC-P

Species reactivity Reacts with: Human

Synthetic peptide within Human S100A4 aa 50 to the C-terminus (C terminal). The exact **Immunogen** 

> sequence is proprietary. Database link: P26447

(Peptide available as ab232852)

Positive control Human tonsil, A549, A375, HeLa and Human small intestine lysates; Human tonsil tissue

**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  $\mathsf{RabMAb}^{\texttt{®}}$  technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

#### **Properties**

**Form** Liquid

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Storage instructions

Stable for 12 months at -20°C.

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Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

**Purity** Protein A purified

Clonality Monoclonal
Clone number EPR2761(2)

**Isotype** IgG

## **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab124805 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
ICC/IF	*** <u>*</u>	1/100 - 1/250.
Flow Cyt (Intra)		1/80 - 1/800.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	★ de de de de (1)	1/1000 - 1/10000. Detects a band of approximately 12 kDa (predicted molecular weight: 12 kDa).
IP		1/10 - 1/100.
IHC-P	<b>★★★★★</b> (4)	1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

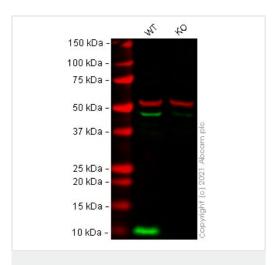
## **Target**

**Tissue specificity** Ubiquitously expressed.

**Sequence similarities** Belongs to the S-100 family.

Contains 2 EF-hand domains.

## **Images**



Western blot - Anti-S100A4 antibody [EPR2761(2)] (ab124805)

**All lanes :** Anti-S100A4 antibody [EPR2761(2)] (ab124805) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2: S100A4 knockout HeLa cell lysate

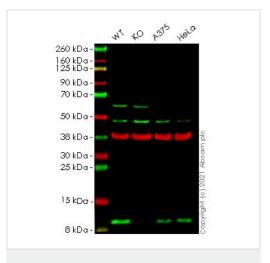
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

**Lanes 1 - 2:** Merged signal (red and green). Green - ab124805 observed at 11 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab124805 was shown to react with S100A4 in wild-type HeLa cells in Western blot with loss of signal observed in S100A4 knockout cell line <a href="mailto:ab265709">ab265709</a> (S100A4 knockout cell lysate <a href="mailto:ab257046">ab257046</a>). 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<sup>®</sup>) before incubation with ab124805 and <a href="mailto:ab7291">ab7291</a> (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 lgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-S100A4 antibody [EPR2761(2)] (ab124805)

**All lanes :** Anti-S100A4 antibody [EPR2761(2)] (ab124805) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate

**Lane 2 :** S100A4 knockout A549 (Human lung carcinoma cell line) whole cell lysate

**Lane 3**: A-375 (Human malignant melanoma cell line) whole cell lysate

**Lane 4 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

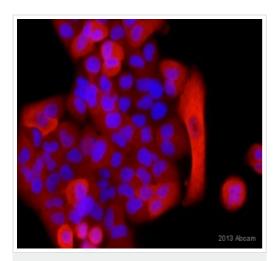
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

**Lanes 1 - 4:** Merged signal (red and green). Green - ab124805 observed at 12 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab124805 was shown to react with S100A4 in wild-type A549 cells in Western blot with loss of signal observed in S100A4 knockout cell line <a href="mailto:ab261865">ab261865</a> (knockout cell lysate <a href="mailto:ab261674">ab261674</a>). Wild-type A549 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 ab124805 and <a href="mailto:ab8245">ab8245</a> (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

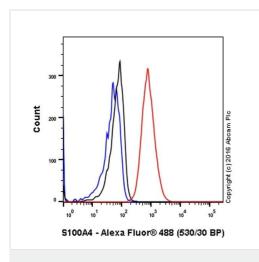


Immunocytochemistry/ Immunofluorescence - Anti-S100A4 antibody [EPR2761(2)] (ab124805)

This image is courtesy of an anonymous Abreview

Unpurified ab124805 staining S100A4 in the A549 cell line from Human lungs by ICC/IF

(Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with Triton X-100 0.25% in PBS. Samples were incubated with primary antibody (1/100) for 45 minutes at 25°C. A TRITC-conjugated Goat anti-rabbit polyclonal was used as the secondary antibody.

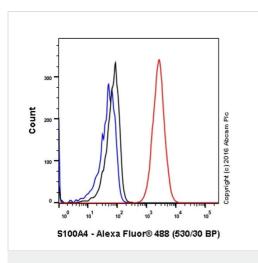


Flow Cytometry (Intracellular) - Anti-S100A4 antibody [EPR2761(2)] (ab124805)

ab124805 staining S100A4 in HeLa (human cervix adenocarcinoma) by intracellular flow cytometry. Cells were fixed with 80% methanoland permeabilised with 0.1% Triton X-100 (in PBS). The sample was incubated with the primary antibody at a dilution of 1/800. A goat anti rabbit lgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: ab172730 rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)

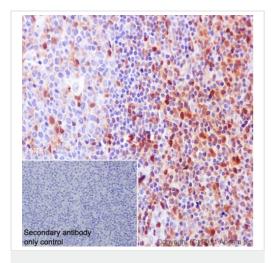


Flow Cytometry (Intracellular) - Anti-S100A4 antibody [EPR2761(2)] (ab124805)

ab124805 staining S100A4 in HeLa (human cervix adenocarcinoma) by intracellular flow cytometry. Cells were fixed with 80% methanoland permeabilised with 0.1% Triton X-100 (in PBS). The sample was incubated with the primary antibody at a dilution of 1/80. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: ab172730 rabbit monoclonal IgG (Black)

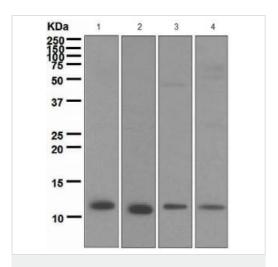
Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-S100A4 antibody
[EPR2761(2)] (ab124805)

ab124805 staining S100A4 in human tonsil tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/500. A goat anti-rabbit IgG H&L (HRP) <u>ab97051</u> was used as the secondary antibody at a dilution of 1/500.

**Negative control 1:** PBS in place of primary antibody.



Western blot - Anti-S100A4 antibody [EPR2761(2)] (ab124805)

**All lanes :** Anti-S100A4 antibody [EPR2761(2)] (ab124805) at 1/1000 dilution (unpurified)

Lane 1 : Human tonsil lysate
Lane 2 : A549 cell lysate
Lane 3 : A375 cell lysate

Lane 4: Human small intestine lysate

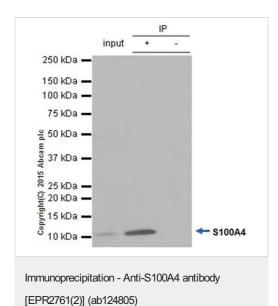
Lysates/proteins at 10 µg per lane.

#### **Secondary**

All lanes: HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 12 kDa

#### Observed band size: 12 kDa



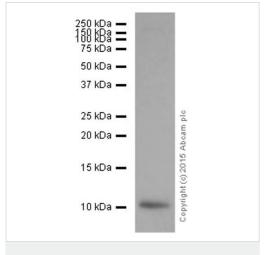
ab124805 immunoprecipitating S100A4. 10 $\mu$ g of cell lysate was incubated with primary antibody at a dilution of 1/30 and Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at a dilution of 1/1500.

Lane 1: Human tonsil whole cell lysate (10ug)

Lane 2: Human tonsil whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab124805

in human tonsil whole cell lysate



Western blot - Anti-S100A4 antibody [EPR2761(2)] (ab124805)

Anti-S100A4 antibody [EPR2761(2)] (ab124805) + A549 (human lung carcinoma) whole cell lysate at 20  $\mu g$ 

#### **Secondary**

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

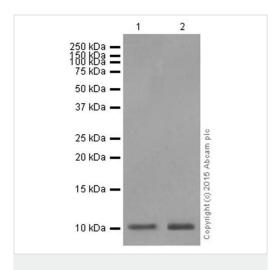
Predicted band size: 12 kDa

Additional bands at: 12 kDa. We are unsure as to the identity of

these extra bands.

Blocking buffer: 5% NFDM /TBST

Diluting Buffer: 5% NFDM /TBST



Western blot - Anti-S100A4 antibody [EPR2761(2)] (ab124805)

**All lanes :** Anti-S100A4 antibody [EPR2761(2)] (ab124805) at 1/2500 dilution

Lane 1: Human tonsil tissue lysate

Lane 2: A375 (human malignant melanoma) whole cell lysate

Lysates/proteins at 10 µg per lane.

## **Secondary**

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

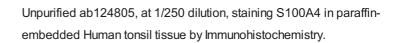
Predicted band size: 12 kDa

Additional bands at: 12 kDa. We are unsure as to the identity of

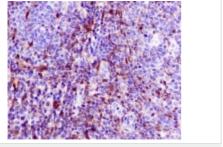
these extra bands.

Blocking Buffer: 5% NFDM /TBST

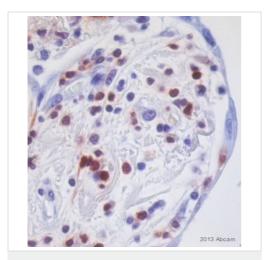
Diluting Buffer: 5% NFDM /TBST



Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-S100A4 antibody
[EPR2761(2)] (ab124805)

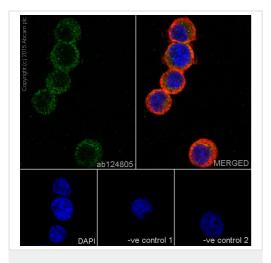


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-S100A4 antibody
[EPR2761(2)] (ab124805)

This image is courtesy of an anonymous Abreview

Immunohistochemical analysis of Human lung tissue, staining S100A4 with unpurified ab124805.

Tissue was fixed with HOPE and blocked with blocking solution for 5 minutes at 25°C. Samples were incubated with primary antibody (1/1000 in diluent) for 1 hour at 25°C. An undiluted HRP-conjugated goat anti-rabbit polyclonal IgG was used as the secondary antibody.

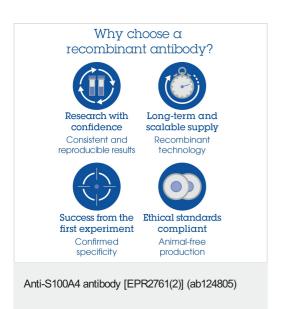


Immunocytochemistry/ Immunofluorescence - Anti-S100A4 antibody [EPR2761(2)] (ab124805)

ab124805 staining S100A4 in Jurkat (human acute T cell leukemia) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at 1/500. ab7291 and ab150120 were used as counterstains for primary antibody ab124805 and secondary antibody ab150077 respectively and DAPI was used as a nuclear counterstain.

**Negative control 1**: Rabbit primary antibody and anti-mouse secondary antibody (ab150120)

**Negative control 2:** Mouse primary antibody (<u>ab7291</u>) and antirabbit secondary antibody (<u>ab150077</u>)



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