abcam

Product datasheet

Anti-WIPI2 antibody [2A2] ab105459

KO VALIDATED

14 References 5 Images

Overview

Product name	Anti-WIPI2 antibody [2A2]
Description	Mouse monoclonal [2A2] to WIPI2
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, IP, IHC-P, IHC-Fr, WB, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide: CSALRLDEDSEHPPMILRTD , corresponding to C terminal amino acids 418-436 of Human WIPI2 (Isoform WIPI2b).
Epitope	EHPPM
Positive control	This antibody gave a positive signal in Human Skeletal Muscle, Human Placenta, Mouse Placenta, Mouse Testis as well as the following whole cell lysates: HeLa, and NIH3T3. IF/ICC: HeLa cells (50mM chloroquine for 24h) Flow Cyt: HeLa cells IHC-P: Human skeletal muscle (normal)
General notes	This antibody clone is manufactured by Abcam. We welcome customer feedback and would appreciate any comments regarding this product and the data presented. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	Preservative: 0.02% Sodium azide Constituent: PBS
Purity	Immunogen affinity purified
Clonality	Monoclonal
Clone number	2A2
Isotype	IgG1

Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab105459** in the following tested applications.

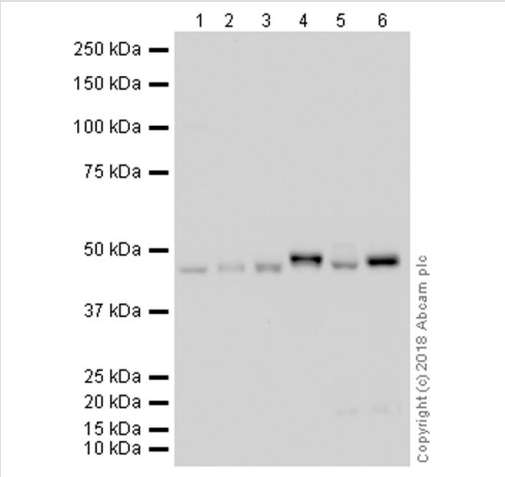
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
IHC-Fr		Use at an assay dependent concentration. Fix with Acetone.
WB		Use at an assay dependent concentration. Predicted molecular weight: 49 kDa.
Flow Cyt		Use 0.1µg for 10^6 cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Target

Function	Probable early component of the autophagy machinery being involved in formation of preautophagosomal structures and their maturation into mature phagosomes in response to PtdIns3P. May bind PtdIns3P.
Tissue specificity	Ubiquitously expressed (at protein level). Highly expressed in heart, skeletal muscle and pancreas. Expression is down-regulated in pancreatic and in kidney tumors.
Sequence similarities	Belongs to the WD repeat SVP1 family. Contains 3 WD repeats.
Cellular localization	Preautophagosomal structure membrane. Enriched at preautophagosomal structure membranes in response to ptdIns3P.

Images



Western blot - Anti-WIPI2 antibody [2A2] (ab105459)

All lanes : Anti-WIPI2 antibody [2A2] (ab105459) at 1 µg/ml

Lane 1 : Human skeletal muscle tissue lysate
Lane 2 : Human placenta tissue lysate
Lane 3 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 4 : NIH 3T3 (Mouse) Whole Cell Lysate
Lane 5 : Pancreas (Mouse) Tissue Lysate
Lane 6 : Testis (Mouse) Tissue Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) (ab65485) at 1/5000 dilution

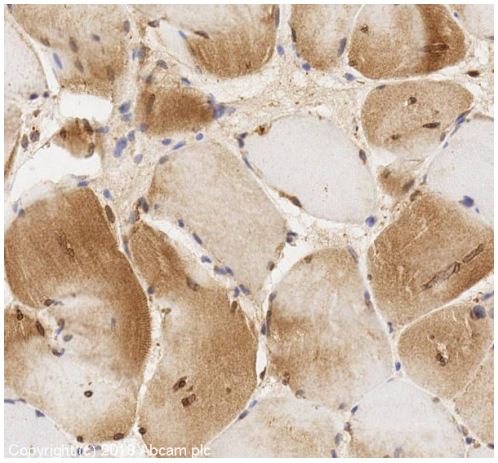
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 49 kDa

Exposure time: 1 minute

Abcam recommends using milk as the blocking agent.

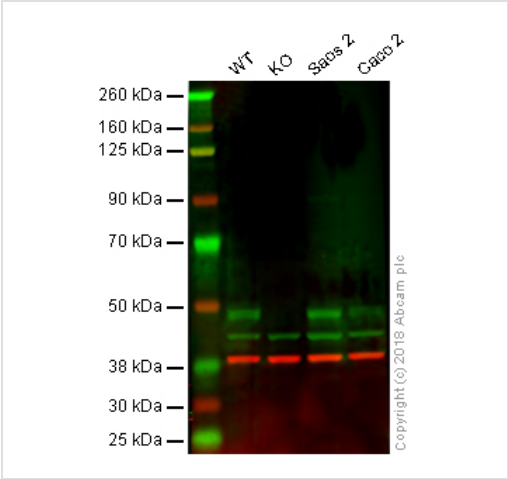


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-WIPI2 antibody [2A2] (ab105459)

IHC image of WIPI2 staining in a section of formalin-fixed paraffin-embedded normal human skeletal muscle* performed on a Leica BOND™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab105459, 1ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*



Western blot - Anti-WIPI2 antibody [2A2] (ab105459)

All lanes : Anti-WIPI2 antibody [2A2] (ab105459) at 1/1000 dilution

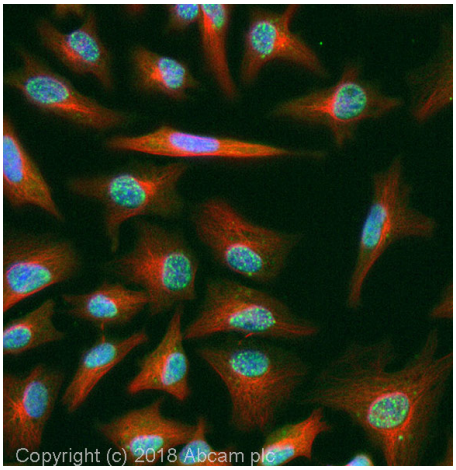
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Wipi2 knockout HAP1 whole cell lysate
Lane 3 : Saos2 whole cell lysate
Lane 4 : CACO2 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 49 kDa
Observed band size: 49 kDa

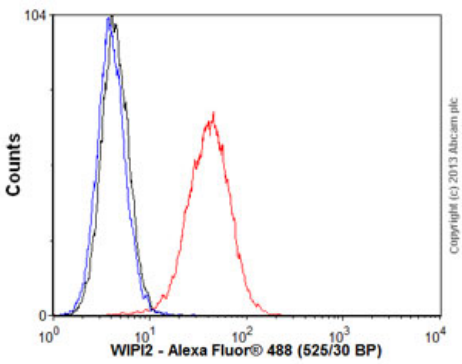
Lanes 1 - 4: Merged signal (red and green). Green - ab105459 observed at 49 kDa. Red - loading control, ab181602, observed at 37 kDa.

ab105459 was shown to recognize WIPI2 in wild-type HAP1 cells as signal was lost at the expected MW in Wipi2 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and Wipi2 knockout samples were subjected to SDS-PAGE. Ab105459 and ab181602 (Rabbit 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed ab216772 and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed ab216777 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-WIPI2 antibody [2A2] (ab105459)

ab105459 staining WIPI2 in HeLa cells. The cells were treated with 50uM chloroquine for 24h and then fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab105459 at 1ugml then detected with an Alexa Fluor® 488 goat anti-rabbit secondary antibody (ab150081) at a 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue), and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at a 1/250 dilution (shown in red).



Flow Cytometry - Anti-WIPI2 antibody [2A2] (ab105459)

Overlay histogram showing HeLa cells stained with ab105459 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab105459, 0.1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H+L) (ab150113) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors