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Product datasheet

Anti-WIPI2 antibody [2A2] ab105459

KO 評価済

4 Abreviews 55 References 画像数 5

製品の概要

製品名	Anti-WIPI2 antibody [2A2]
製品の詳細	Mouse monoclonal [2A2] to WIPI2
由来種	Mouse
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, IHC-P, WB
種交差性	**交差種:** Mouse, Human
免疫原	Synthetic peptide corresponding to Human WIPI2 aa 400-500 (C terminal).
エピトープ	EHPPM
ポジティブ・コントロール	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 (Intra): HeLa cells IHC-P: Human skeletal muscle (normal).
特記事項	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
バッファー	Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
精製度	Protein G purified

ポリ/モノ	モノクローナル
クローン名	2A2
アイソタイプ	IgG1
軽鎖の種類	kappa

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab105459の使用に適用されます

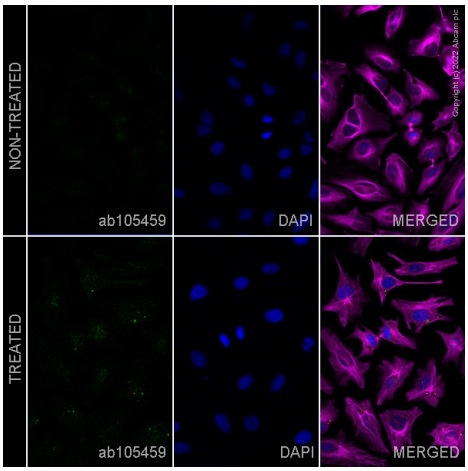
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use 0.1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (3)	Use at an assay dependent concentration.
IHC-P	★★★★☆ (1)	Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 49 kDa.

ターゲット情報

機能	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.
組織特異性	Ubiquitously expressed (at protein level). Highly expressed in heart, skeletal muscle and pancreas. Expression is down-regulated in pancreatic and in kidney tumors.
配列類似性	Belongs to the WD repeat SVP1 family. Contains 3 WD repeats.
細胞内局在	Preautophagosomal structure membrane. Enriched at preautophagosomal structure membranes in response to ptdIns3P.

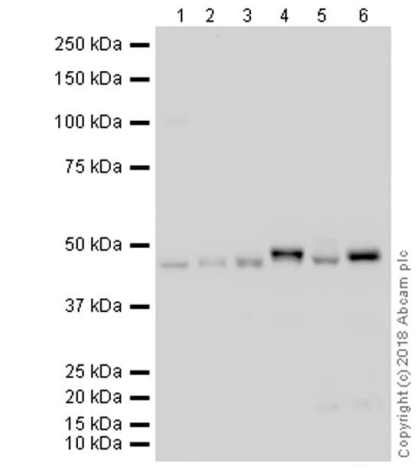
画像



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

ab105459 staining WIPI2 in HeLa Chloroquine treated cells. The cells were fixed with 100% methanol 5 min), permeabilized with 0.1% PBS-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 5µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L Alexa Fluor® 488) preadsorbed at 1/1000 dilution shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L Alexa Fluor® 594) at 1/1000 dilution shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI shown in blue).

Image was acquired with a high-content analyser Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



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

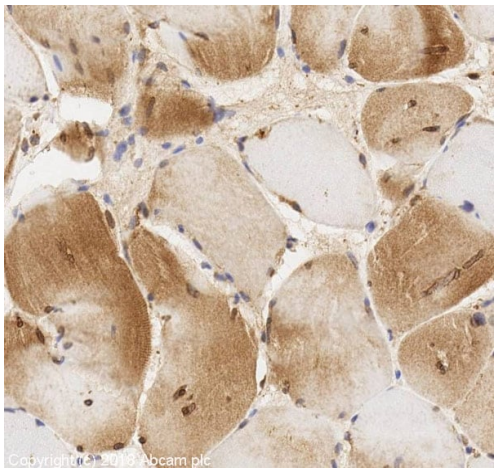
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 49 kDa

Exposure time: 1 minute

Abcam recommends using milk as the blocking agent.

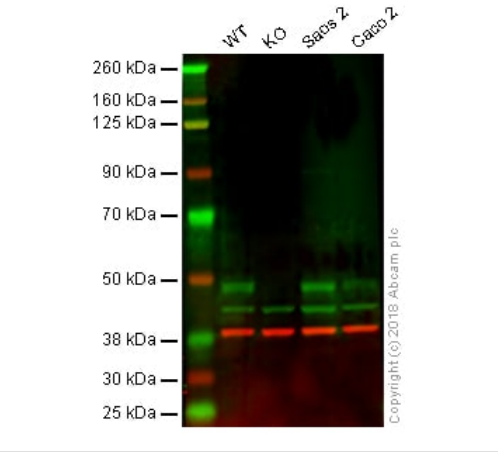


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-WIPI2 antibody [2A2] (ab105459)

IHC image of WIPI2 staining in a section of formalin-fixed paraffin-embedded normal human skeletal muscle* performed on a Leica BOND^{TM} 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

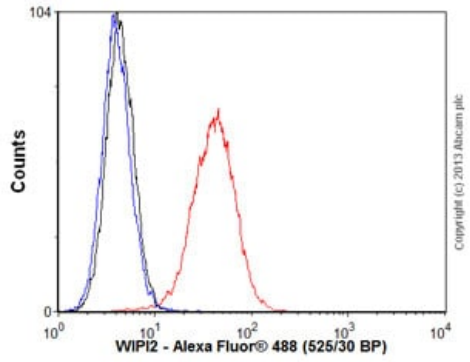
Lysates/proteins at 20 µg per lane.

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.



Flow Cytometry (Intracellular) - 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.

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