abcam

ab66340

Anti-AMF antibody [1B7D7]

KO Validated

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	Preservative: 0.05% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human GPI. The exact immunogen used to generate this antibody is proprietary information. Database link P06744
Clone number	1B7D7
Purification technique	Affinity purification Protein G
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purified from TCS.

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1 µg/mL
Notes	

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

Flow Cyt

Tested

Species	Human
Dilution info	1/100
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	1/500 - 1/5000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/200 - 1/1000
Notes	-

Target data

See full target information GPI

Function Isomerase that catalyzes the conversion of alpha-D-glucose-6-phosphate to beta-D-fructose-6-phosphate, the second step in glycolysis, and the reverse reaction in gluconeogenesis, within the cytoplasm (PubMed:28803808). Also shows C2-epimerase activity, interconverting D-glucose-6-phosphate (G6P) and D-mannose-6-phosphate (M6P) (By similarity). Also displays anomerase activity, interconverting alpha and beta-anomeric forms of G6P, D-fructose-6-phosphate and M6P (By similarity). In addition to its metabolic role, this enzyme functions extracellularly as a cytokine: acts as autocrine motility factor (AMF), a secreted angiogenic factor that enhances endothelial cell motility (PubMed:11437381). Functions as neuroleukin, a neurotrophic factor supporting the survival of spinal and sensory

neurons (PubMed:11004567, PubMed:3352745). Released by lectin-stimulated T-cells to induce immunoglobulin secretion (PubMed:11004567, PubMed:3352745).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

This product was changed from ascites to supernatant. Lot no's high than GR185888-22 are from Tissue Culture Supernatant.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

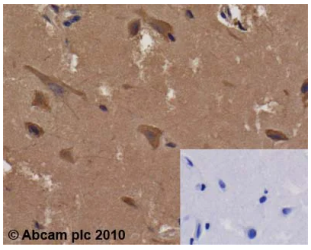
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

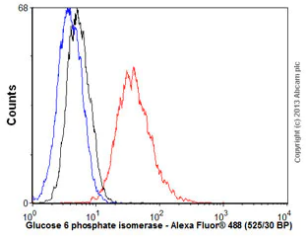
5 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMF antibody [1B7D7] (ab66340)

AMF Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-AMF antibody

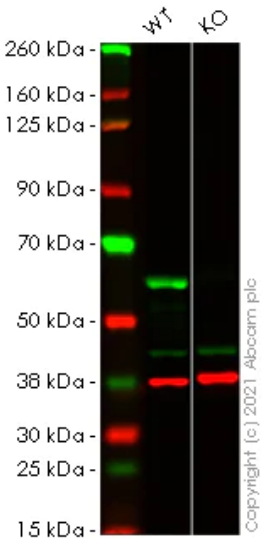
ab66340 (1µg/ml) staining AMF in human cerebral cortex using an automated system (DAKO Autostainer Plus). Using this protocol there is strong cytoplasmic staining of neurons and of the neuropil.
Inset panel depicts negative control (no primary antibody).
Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.</



Flow Cytometry - Anti-AMF antibody [1B7D7] (ab66340)

AMF Flow Cytometry staining using mouse Anti-AMF antibody

Overlay histogram showing HepG2 cells stained with ab66340 (red line). The cells were fixed with 4% paraformaldehyde (10 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 (ab66340, 1/100 dilution) 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 HepG2 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Western blot - Anti-AMF antibody [1B7D7] (ab66340)

AMF Western blot staining using mouse Anti-AMF antibody

Lanes 1 - 2: Merged signal (red and green). Green - ab66340 observed at 63 kDa. Red - loading control ab181602 (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37 kDa.
ab66340 was shown to react with AMF in wild-type HEK-293T cells in Western blot with loss of signal observed in GPI knockout cell line ab266834 (GPI knockout cell lysate ab257458). Wild-type HEK-293T and GPI knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab66340 and ab181602 (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 h at room temperature before imaging.

All lanes:
Western blot - Anti-AMF antibody [1B7D7] (ab66340) at 1/1000 dilution

Lane 1:
Wild-type HEK-293T cell lysate at 20 µg

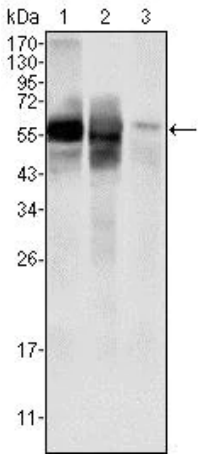
Lane 2:
GPI knockout HEK-293T cell lysate at 20 µg

Lane 2:
Western blot - Human GPI (AMF) knockout HEK-293T cell line (ab266834)

Performed under reducing conditions.

Predicted band size: 63 kDa

Observed band size: 63 kDa



Western blot - Anti-AMF antibody [1B7D7] (ab66340)

AMF Western blot staining using mouse Anti-AMF antibody

All lanes:
Western blot - Anti-AMF antibody [1B7D7] (ab66340) at 1/2000 dilution

Lane 1:
Cell lysates prepared from HepG2 cells at 100 µg

Lane 2:
Cell lysates prepared from SMMC-7721 cells at 100 µg

Secondary

All lanes:
HRP-conjugated Goat polyclonal to mouse IgG1

Predicted band size: 63 kDa

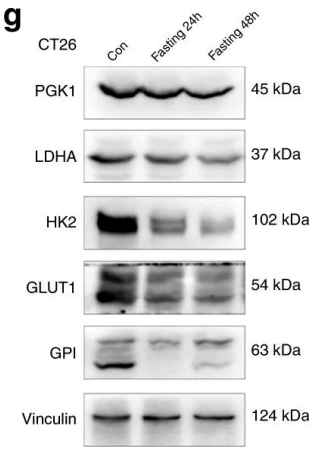


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Western blot - Anti-AMF antibody [1B7D7] (ab66340)

AMF Western blot staining using mouse Anti-AMF antibody

AMF western blot using anti-AMF antibody [1B7D7] ab66340. Publication image and figure legend from Weng, M. L., Chen, W. K., et al., 2020, Nat Commun, PubMed 32313017.

ab66340 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab66340 please see the product overview.

Fasting impairs glycolysis and proliferation of CT26 cells in vitro and in vivo.Fasting inhibited CT26 cell proliferation as measured by a CCK8 assay (from left to right: P = 0.0009; P = 0.0006). b Cell proliferation was also evaluated using EdU immunofluorescence staining. Proliferating cells were labeled with EdU. n = 3; scale bar: 100 μm. c Bioinformatics analysis of differentially expressed genes identified via iTRAQ proteomics. We magnified the genes related to the glycolysis pathway particularly. d The graph shows the relative fold fraction of EdU-positive cells. e Fasting reduced glucose uptake in CT26 cells. f Fasting decreased lactate production via glycolysis in CT26 cells. g Fasting downregulated the expression of rate-limiting glycolytic enzymes in glucose metabolism (GLUT1, HK2, LDHA, PGK1, and GPI) by western blot. h ECAR, an indicator of glycolysis, was reduced in CT26 cell cultured in the fasting mimic medium. i OCR, which reflects mitochondrial respiration, was increased in CT26 cell cultured in the fasting mimic medium. j CT26 cells were injected into BALB/c mice. When the tumors were palpable, the mice were randomly assigned to the control group or the fasting mimic diet (FMD) group. Photograph of dissected tumors (upper: FMD group; lower: control group; n = 5). k Fasting downregulated the transcription of rate-limiting glycolytic enzymes in glucose metabolism (GLUT1, HK2, LDHA, PGK1, and GPI) by qRT-PCR. l The tumor volumes were measured every day after the 9th day. The FMD attenuated tumor growth in mice (n = 5). m, o Tumor weights and tumor volumes on the 25th day (n = 5; P = 0.001; P = 0.0292). n Representative 18F-FDG microPET/CT imaging of tumor-bearing mice (n = 3) (upper: control group; lower: FMD group). The tumors are indicated with arrows. p The ratio of the tumor SUVmax in the control group and the FMD group (n = 3; P = 0.0459). Error bars, mean ± SD, the data are from three independent experiments. Two-sided t tests. *p < 0.05, **p < 0.01, ***p < 0.001, compared with the control group.

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