abcam

Product datasheet

Anti-AMF antibody [1B7D7] ab66340

KO VALIDATED

6 Abreviews 17 References 5 Images

Overview

Product name	Anti-AMF antibody [1B7D7]
Description	Mouse monoclonal [1B7D7] to AMF
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, IHC-P, WB, Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment corresponding to Human AMF.
Positive control	WB: HEK293T, HepG2 and SMMC-7721 cell lysate. Flow Cyt: HepG2 cells. ICC: L-02 cells. IHC-P: Human cerebral cortex tissue.
General notes	This product was changed from ascites to supernatant. Lot no's high than GR185888-22 are from Tissue Culture Supernatant. 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

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: 0.05% Sodium azide Constituent: PBS
Purity	Protein G purified
Purification notes	Purified from TCS.
Clonality	Monoclonal
Clone number	1B7D7

Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab66340 in the following tested applications.

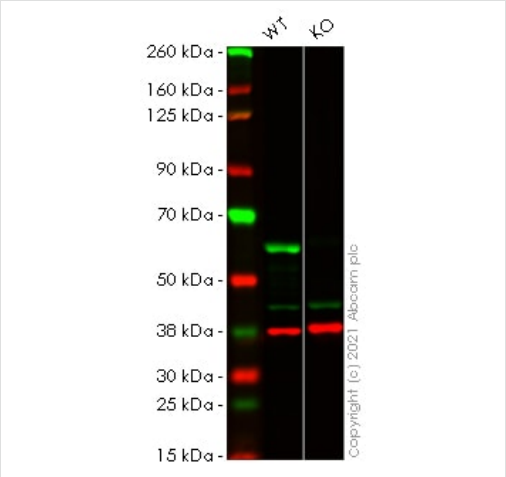
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★☆ (1)	1/200 - 1/1000.
IHC-P	★★★★☆ (1)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	★★★★☆ (2)	1/500 - 1/5000. Predicted molecular weight: 63 kDa.
Flow Cyt		1/100. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Target

Function	Besides it's role as a glycolytic enzyme, mammalian GPI can function as a tumor-secreted cytokine and an angiogenic factor (AMF) that stimulates endothelial cell motility. GPI is also a neurotrophic factor (Neuroleukin) for spinal and sensory neurons.
Pathway	Carbohydrate degradation; glycolysis; D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose: step 2/4.
Involvement in disease	Defects in GPI are the cause of hemolytic anemia non-spherocytic due to glucose phosphate isomerase deficiency (HA-GPID) [MIM:613470]. It is a form of anemia in which there is no abnormal hemoglobin or spherocytosis. It is caused by glucose phosphate isomerase deficiency. Severe GPI deficiency can be associated with hydrops fetalis, immediate neonatal death and neurological impairment.
Sequence similarities	Belongs to the GPI family.
Post-translational modifications	Phosphorylation at Ser-185 by CK2 has been shown to decrease enzymatic activity and may contribute to secretion by a non-classical secretory pathway. ISGylated.
Cellular localization	Cytoplasm. Secreted.

Images



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

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

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : GPI knockout HEK-293T cell lysate

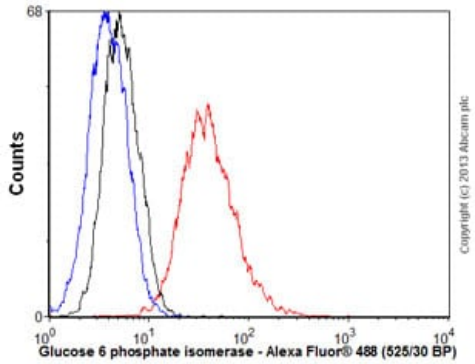
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 63 kDa
Observed band size: 63 kDa

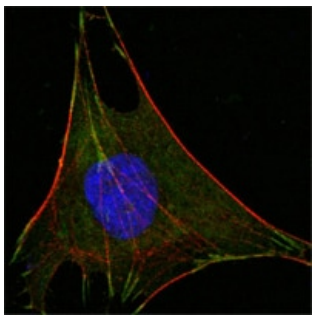
Lanes 1 - 2: Merged signal (red and green). Green - ab66340 observed at 63 kDa. Red - loading control **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37 kDa.

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.



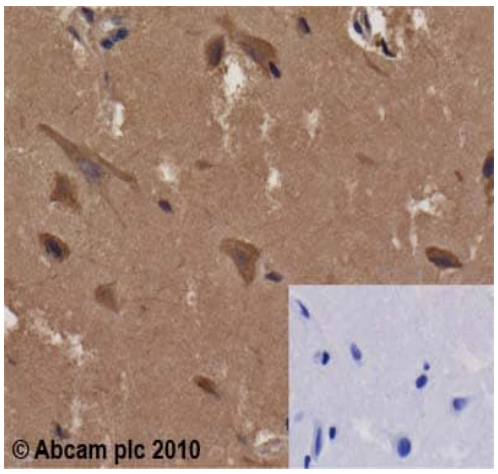
Flow Cytometry - Anti-AMF antibody [1B7D7] (ab66340)

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.



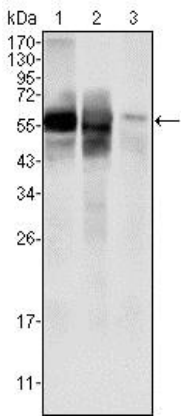
Immunocytochemistry/ Immunofluorescence - Anti-AMF antibody [1B7D7] (ab66340)

ab66340 at 1000 dilution staining AMF in L-02 cells by Immunocytochemistry/ Immunofluorescence. An Alexa Fluor® 488 conjugated Goat polyclonal to mouse IgG1 was used as secondary antibody. Green staining in image show positive staining with ab66340, actin filaments were stained red with DY-554 phalloidin and nuclei stained blue with DRAQ5 fluorescent DNA dye.



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

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.



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

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

Lane 1 : Cell lysates prepared from HepG2 cells.
Lane 2 : Cell lysates prepared from SMMC-7721 cells.

Lysates/proteins at 100 µg per lane.

Secondary
All lanes : HRP-conjugated Goat polyclonal to mouse IgG1

Predicted band size: 63 kDa

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