abcam

ab313589

Anti-AMF antibody [EPR28372-33]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR28372-33
Purification technique	Affinity purification Protein A
Concentration	0.527 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/5000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Mouse
Dilution info	1/5000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/5000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Species	Mouse
Dilution info	1/30
Notes	-

Species	Rat
Dilution info	1/30
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/5000
Notes	-

Species	Mouse
Dilution info	1/5000
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
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:2344351, PubMed:7277315, PubMed:8417789). 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 (By similarity). Functions as neuroleukin, a neurotrophic factor supporting the survival of spinal and sensory neurons (PubMed:3352745, PubMed:3764429). Released by lectin-stimulated T-cells to induce immunoglobulin secretion (PubMed:3352745).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
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

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

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.

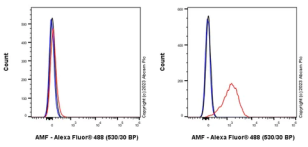
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

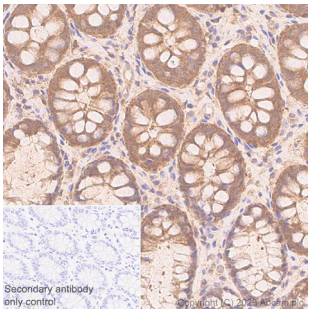
---

16 product images



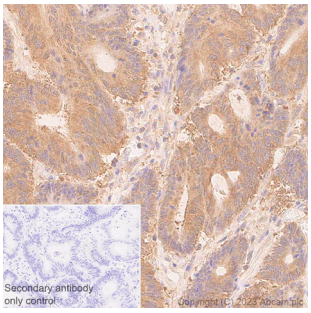
Flow Cytometry (Intracellular) - Anti-AMF antibody [EPR28372-33] (ab313589)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized AMF KO HEK293T (human AMF knockout embryonic kidney epithelial cell, Left) / Parental HEK293T (Right) cells labelling AMF with ab313589 at 1/5000 dilution (0.01 ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



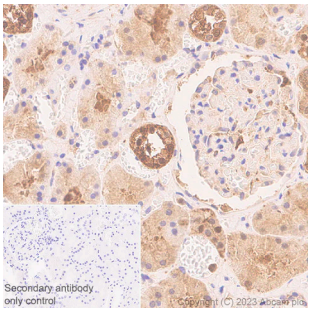
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMF antibody [EPR28372-33] (ab313589)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling AMF with ab313589 at 1/5000 (0.105 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human colon. The section was incubated with ab313589 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 10 mins.



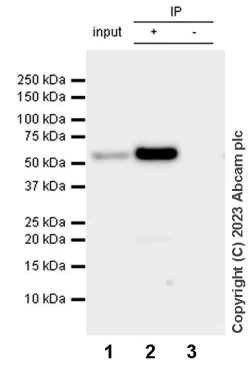
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMF antibody [EPR28372-33] (ab313589)

Immunohistochemical analysis of paraffin-embedded human colon carcinom tissue labeling AMF with ab313589 at 1/5000 (0.105 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human colon carcinoma. The section was incubated with ab313589 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 10 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMF antibody [EPR28372-33] (ab313589)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling AMF with ab313589 at 1/5000 (0.105 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human kidney. The section was incubated with ab313589 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 10 mins.



Immunoprecipitation - Anti-AMF antibody [EPR28372-33] (ab313589)

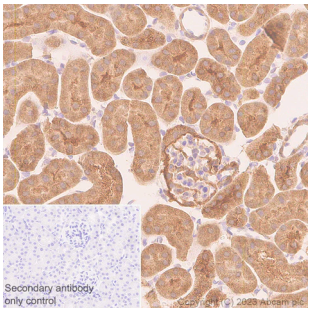
AMF was immunoprecipitated from 0.35 mg 293T (human embryonic kidney epithelial cell) whole cell lysate with ab313589 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab313589 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution. Lane 1: 293T (human embryonic kidney epithelial cell) whole cell lysate Lane 2: 293T (human embryonic kidney epithelial cell) whole cell lysate Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab313589 in 293T whole cell lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 8 seconds
All lanes:
Immunoprecipitation - Anti-AMF antibody [EPR28372-33] (ab313589) at 1/30 dilution

All lanes:
293T (human embryonic kidney epithelial cell) whole cell lysate

Secondary

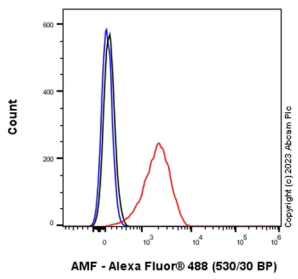
All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 8s



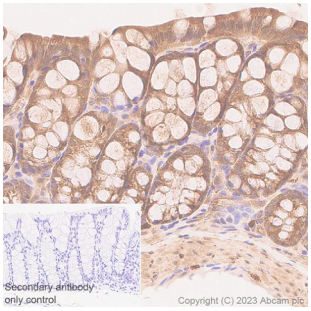
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMF antibody [EPR28372-33] (ab313589)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling AMF with ab313589 at 1/5000 (0.105 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on mouse kidney. The section was incubated with ab313589 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 10 mins.



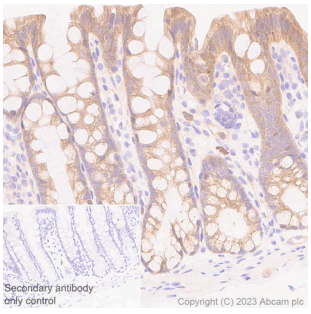
Flow Cytometry (Intracellular) - Anti-AMF antibody [EPR28372-33] (ab313589)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling AMF with ab313589 at 1/5000 dilution (0.01 ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



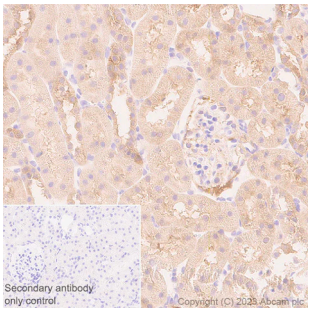
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMF antibody [EPR28372-33] (ab313589)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling AMF with ab313589 at 1/5000 (0.105 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on mouse colon. The section was incubated with ab313589 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 10 mins.



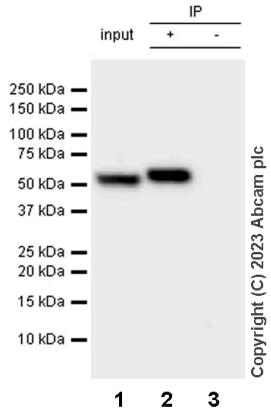
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMF antibody [EPR28372-33] (ab313589)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling AMF with ab313589 at 1/5000 (0.105 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on rat colon. The section was incubated with ab313589 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 10 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-AMF antibody [EPR28372-33] (ab313589)

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling AMF with ab313589 at 1/5000 (0.105 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on rat kidney. The section was incubated with ab313589 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Incubate slides with 3% Hydrogen Peroxide for 10 mins at room temperature after secondary antibody incubation to reduce the background. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 10 mins.



Immunoprecipitation - Anti-AMF antibody [EPR28372-33] (ab313589)

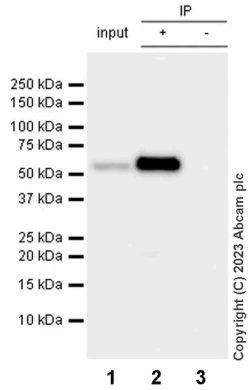
AMF was immunoprecipitated from 0.35 mg Rat brain tissue lysate with ab313589 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab313589 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution. Lane 1: Rat brain tissue lysate Lane 2: Rat brain tissue lysate Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab313589 in rat brain tissue lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 15 seconds
All lanes:
Immunoprecipitation - Anti-AMF antibody [EPR28372-33] (ab313589) at 1/30 dilution

All lanes:
Rat brain tissue lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 15s



Immunoprecipitation - Anti-AMF antibody [EPR28372-33] (ab313589)

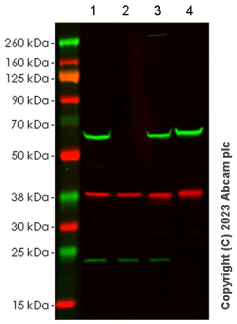
AMF was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast) whole cell lysate with ab313589 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab313589 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution. Lane 1: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate Lane 2: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab313589 in NIH/3T3 whole cell lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 8 seconds
All lanes:
Immunoprecipitation - Anti-AMF antibody [EPR28372-33] (ab313589) at 1/30 dilution

All lanes:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 8s



Western blot - Anti-AMF antibody [EPR28372-33] (ab313589)

Blocking and diluting buffer and concentration: 5% NFDM/TBST Lysates at 20 µg per lane. The samples were run on a Bis-Tris gel under reducing conditions. Western blot: Anti-AMF antibody (ab313589) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody 6C5 (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab313589 was shown to bind specifically to AMF. Target of interest was observed at 62 kDa in wild-type HEK293T cell lysates (lane 1) with no signal observed at this size in GPI (AMF) knockout cell line ab266834 (knockout cell lysate ab257458) (lane 2). To generate this image, samples were first run on an SDS-PAGE gel then transferred onto an immobilon-FL PVDF membrane. Membranes were blocked in a fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed in TBS-T, incubated with secondary antibodies Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution for 1 h at room temperature, washed again then imaged. The identity of the lower MW band at approximately 20 kDa is unknown.
All lanes:
Western blot - Anti-AMF antibody [EPR28372-33] (ab313589) at 1/1000 dilution

Lane 1:
Wild-type HEK293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 2:
GPI (AMF) knockout HEK293T whole cell lysate at 20 µg

Lane 3:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

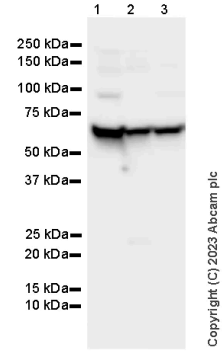
Lane 4:
Mouse brain tissue lysate at 20 µg

Secondary

Lanes 1 - 4:
Goat Anti-Rabbit IgG H&L (800CW) at 1/20000 dilution

Lanes 1 - 4:
Goat Anti-Mouse IgG H&L (680RD) at 1/20000 dilution

Observed band size: 62 kDa



Western blot - Anti-AMF antibody [EPR28372-33] (ab313589)

Blocking and diluting buffer and concentration: 5% NFDM/TBST Exposure time: 1 seconds
All lanes:
Western blot - Anti-AMF antibody [EPR28372-33] (ab313589) at 1/1000 dilution

Lane 1:
Human cerebellum tissue lysate at 20 µg

Lane 2:
Human liver tissue lysate at 20 µg

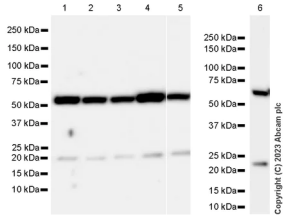
Lane 3:
Human kidney tissue lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 62 kDa

Exposure time: 1s



Western blot - Anti-AMF antibody [EPR28372-33] (ab313589)

Blocking and diluting buffer and concentration: 5% NFDM/TBST The identity of the lower MW band at approximately 20 kDa is unknown. Exposure time: Lane 1-5: 10 seconds; Lane 6: 26 seconds
All lanes:
Western blot - Anti-AMF antibody [EPR28372-33] (ab313589) at 1/1000 dilution

Lane 1:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 3:
PC-3 (human prostate adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
TF-1 (human erythroleukemia erythroblast) whole cell lysate at 20 µg

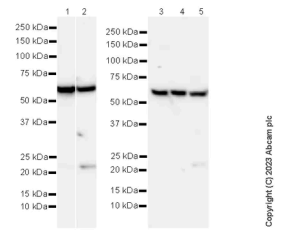
Lane 5:
Neuro-2a (mouse neuroblastoma neuroblast) whole cell lysate at 20 µg

Lane 6:
C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 62 kDa



Western blot - Anti-AMF antibody [EPR28372-33] (ab313589)

Blocking and diluting buffer and concentration: 5% NFDM/TBST The identity of the lower MW band at approximately 20 kDa is unknown. Exposure time: 10 seconds
All lanes:
Western blot - Anti-AMF antibody [EPR28372-33] (ab313589) at 1/1000 dilution

Lane 1:
Mouse liver tissue lysate at 20 µg

Lane 2:
Mouse spleen tissue lysate at 20 µg

Lane 3:
Rat brain tissue lysate at 20 µg

Lane 4:
Rat liver tissue lysate at 20 µg

Lane 5:
Rat kidney tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 62 kDa

Exposure time: 10s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.