abcam

Product datasheet

Anti-GCLC antibody [EPR20078] ab207777

Recombinant RabMAb

7 References 7 Images

Overview

Product name	Anti-GCLC antibody [EPR20078]						
Description	Rabbit monoclonal [EPR20078] to GCLC						
Host species	Rabbit						
Tested applications	Suitable for: Flow Cyt (Intra), IP, ICC/IF, WB						
Species reactivity	Reacts with: Mouse, Rat, Human						
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.						
Positive control	WB: HepG2, MCF7, A549 C6 PC-12 and RAW 264.7 whole cell lysates; human lung and fetal liver lysates; rat liver lysate; mouse liver and spleen lysates. ICC/IF: HpeB2 and PC-12 cells. Flow Cyt (intra): PC-12 cells. IP: HepG2 whole cell lysate.						
General notes	 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 production For more information <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>. 						

Properties							
Form	Liquid Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle. pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA						
Storage instructions							
Storage buffer							
Purity	Protein A purified						
Clonality	Monoclonal						
Clone number	EPR20078						

Applications

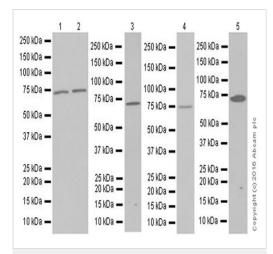
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab207777 in the following tested applications.

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

Application	Abreviews	Notes
Flow Cyt (Intra)		1/60.
IP		1/40.
ICC/IF		1/100.
WB		1/1000. Detects a band of approximately 73 kDa (predicted molecular weight: 73 kDa).

Target							
Pathway	Sulfur metabolism; glutathione biosynthesis; glutathione from L-cysteine and L-glutamate: step 1/2.						
Involvement in disease	Defects in GCLC are the cause of hemolytic anemia (HAGGSD) [MIM:230450].						
Sequence similarities	Belongs to the glutamatecysteine ligase type 3 family.						

Images



Western blot - Anti-GCLC antibody [EPR20078] (ab207777) Lanes 1-2 : Anti-GCLC antibody [EPR20078] (ab207777) at 1/2000 dilution Lanes 3-5 : Anti-GCLC antibody [EPR20078] (ab207777) at 1/1000 dilution

Lane 1 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 2 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lane 3: A549 (Human lung carcinoma cell line) whole cell lysate

Lane 4 : Human lung lysate

Lane 5 : Human fetal liver lysate

Lysates/proteins at 10 µg per lane.

Secondary

Lanes 1-3 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at

1/100000 dilution

Lanes 4-5 : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 73 kDa Observed band size: 73 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

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Western blot - Anti-GCLC antibody [EPR20078] (ab207777) Lanes 1-2 : Anti-GCLC antibody [EPR20078] (ab207777) at 1/2000 dilution

Lanes 3-6 : Anti-GCLC antibody [EPR20078] (ab207777) at 1/1000 dilution

Lane 1 : C6 (Rat glial tumor cell line) whole cell lysate Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma cell line)

whole cell lysate

Lane 3 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate

Lane 4 : Rat liver lysate

Lane 5 : Mouse liver lysate

Lane 6 : Mouse spleen lysate

Lysates/proteins at 10 µg per lane.

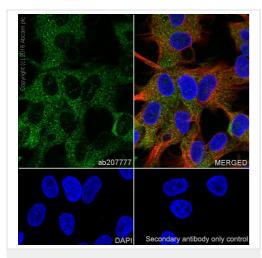
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

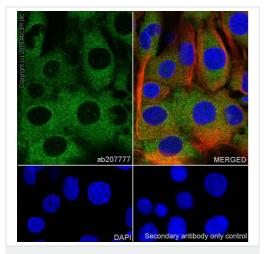
Predicted band size: 73 kDa Observed band size: 73 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-3: 3 minutes; Lane 4: 15 seconds; Lane 5: 3 seconds; Lane 6: 5 seconds.



Immunocytochemistry/ Immunofluorescence - Anti-GCLC antibody [EPR20078] (ab207777)



Immunocytochemistry/ Immunofluorescence - Anti-GCLC antibody [EPR20078] (ab207777)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling GCLC with ab207777 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic and weak nuclear staining on HepG2 cell line. The nuclear counterstain is DAPI (blue).

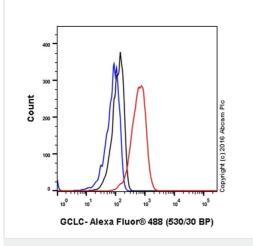
Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] -Microtubule Marker (Alexa Fluor[®] 594) (<u>ab195889</u>) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (**ab150077**) at 1/1000 dilution.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized PC-12 (Rat adrenal gland pheochromocytoma cell line) cells labeling GCLC with ab207777 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic and weak nuclear staining on PC-12 cell line. The nuclear counterstain is DAPI (blue).

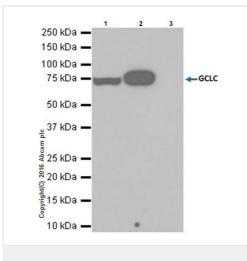
Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (ab195889) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) at 1/1000 dilution.



Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed PC-12 (Rat adrenal gland pheochromocytoma cell line) cells labeling GCLC with ab207777 at 1/60 dilution (red) compared with aRabbit IgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) at 1/2000 dilution was used as the secondary antibody.

Flow Cytometry (Intracellular) - Anti-GCLC antibody [EPR20078] (ab207777)



Immunoprecipitation - Anti-GCLC antibody [EPR20078] (ab207777) GCLC was immunoprecipitated from 0.35 mg of HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate with ab207777 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab207777 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/1000 dilution.

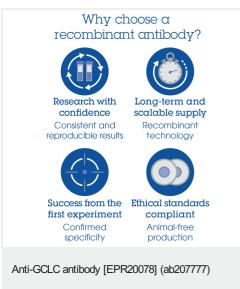
Lane 1: HepG2 whole cell lysate 10µg (Input).

Lane 2: ab207777 IP in HepG2 whole cell lysate.

Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) instead of ab207777 in HepG2 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.



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