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GCLC Polyclonal Antibody

Catalog Number **PA5-19702**

Product data sheet

Details	
Size	100 µg
Host/Isotope	Rabbit / IgG
Class	Polyclonal
Type	Antibody
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 50 - 150 of Human GCLC.
Conjugate	Unconjugated
Form	Liquid
Concentration	0.9 mg/mL
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.

Species Reactivity	
Species reactivity	Human, Mouse, Rat
Published species	Human

Tested Applications	Dilution *
Western Blot (WB)	1 µg/mL
Immunocytochemistry (ICC/IF)	5 µg/mL

Published Applications	
Western Blot (WB)	See 2 publications below
Miscellaneous PubMed (Misc)	See 1 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

This antibody is predicted to react with cow based on sequence homology.

Background/Target Information

Glutamate-cysteine ligase, also known as gamma-glutamylcysteine synthetase is the first rate-limiting enzyme of glutathione synthesis. The enzyme consists of two subunits, a heavy catalytic subunit and a light regulatory subunit. This locus encodes the catalytic subunit, while the regulatory subunit is derived from a different gene located on chromosome 1p22-p21. Mutations at this locus have been associated with hemolytic anemia due to deficiency of gamma-glutamylcysteine synthetase and susceptibility to myocardial infarction.

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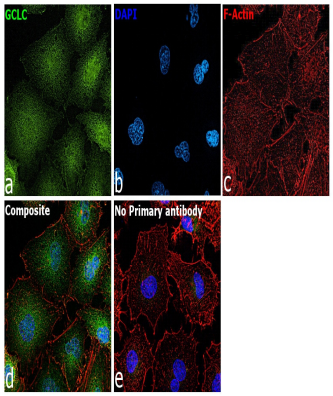
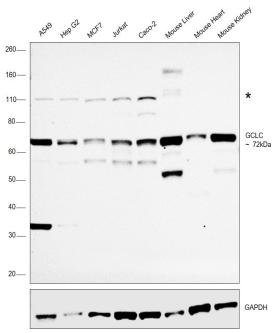
Product Images For GCLC Polyclonal Antibody



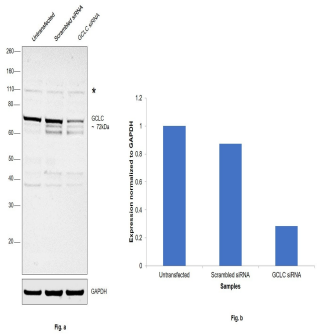
GCLC Antibody (PA5-19702) in ICC/IF

Immunofluorescence analysis GCLC Polyclonal Antibody was performed using 70% confluent log phase A549 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 1 hour at room temperature. The cells were labeled with GCLC Polyclonal Antibody (Product # PA5-19702) at 5 µg/mL in 0.1% BSA, incubated at 4 degree Celsius overnight and then with Goat anti-Rabbit IgG (H+L) Superclonal™ Recombinant Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing cytosolic localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



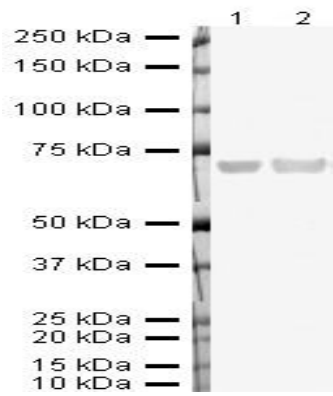
GCLC Antibody (PA5-19702) in WB

Western blot was performed using Anti-GCLC Polyclonal Antibody (Product # PA5-19702) and a 72 kDa band corresponding to GCLC was observed across cell lines tested along with an uncharacterized band at ~110 kDa. Whole cell extracts (30 µg lysate) of A549 (Lane 1), Hep G2 (Lane 2), MCF7 (Lane 3), Jurkat (Lane 4), Caco-2 (Lane 5), Mouse Liver (Lane 6), Mouse Heart (Lane 7) and Mouse Kidney (Lane 8) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1ug/ml) and detected by chemiluminescence with Goat anti-Rabbit IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).



GCLC Antibody (PA5-19702) in WB

Knockdown of GCLC was achieved by transfecting Hep G2 with GCLC specific siRNAs (Silencer® select Product # s5800). Western blot analysis (Fig. a) was performed using whole cell extracts from the GCLC knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with GCLC Polyclonal Antibody (Product # PA5-19702, 1ug/ml) and Goat anti-Rabbit IgG (H+L), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 0.25µg/ml, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to GCLC. An uncharacterized band (*) at ~110 kDa was observed in the samples.



GCLC Antibody (PA5-19702) in WB

Western blot analysis of Human Skeletal Muscle Tissue Lysate using Product # PA5-19702, GCLC primary antibody at a dilution of 1 µg/mL (lane 1). Staining of Human Kidney Tissue Lysate at a dilution of 1 µg/mL (lane 2). Blot treated with a secondary HRP-conjugated Goat polyclonal anti-Rabbit antibody was used at a dilution of 1:3000.

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PubMed References For GCLC Polyclonal Antibody

2 Western Blot References

Species / Dilution	Summary
Human / Not Cited	PA5-19702 was used in Western Blotting to suggest histone methyltransferase G9a as a promising target for overcoming cisplatin resistance in head and neck squamous cell carcinoma. Molecular cancer therapeutics (2017; 16: 1421) **"Histone Methyltransferase G9a Drives Chemotherapy Resistance by Regulating the Glutamate-Cysteine Ligase Catalytic Subunit in Head and Neck Squamous Cell Carcinoma."** Author(s):Liu CW,Hua KT,Li KC,Kao HF,Hong RL,Ko JY,Hsiao M,Kuo ML,Tan CT PubMed Article URL:http://dx.doi.org/10.1158/1535-7163.MCT-16-0567-T
Human / 1:1000	PA5-19702 was used in western blot to study the epigenetic regulation of oxidative genes involved in the pathogenesis of chronic obstructive pulmonary disease Chest (2016; 149: 474) **"Cigarette Smoke-Induced Hypermethylation of the GCLC Gene Is Associated With COPD."** Author(s):Cheng L,Liu J,Li B,Liu S,Li X,Tu H PubMed Article URL:http://dx.doi.org/10.1378/chest.14-2309

1 Miscellaneous PubMed References

Species / Dilution	Summary
Human / Not Cited	PA5-19702 was used in western blot to test if erythroid 2-like 2 regulates expression of ITPR3 Gastroenterology (2015; 149: 211) **"Nuclear Factor, Erythroid 2-Like 2 Regulates Expression of Type 3 Inositol 1,4,5-Trisphosphate Receptor and Calcium Signaling in Cholangiocytes."** Author(s):Weerachayaphorn J,Amaya MJ,Spirli C,Chansela P,Mitchell-Richards KA,Ananthanarayanan M,Nathanson MH PubMed Article URL:http://dx.doi.org/10.1053/j.gastro.2015.03.014

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