

Anti-GAA Antibody Picoband™ (monoclonal, 2G7)

Catalog # ABO14926

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P10253
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-GAA Antibody Picoband™ (monoclonal, 2G7) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	2548
Other Names	Lysosomal alpha-glucosidase, 3.2.1.20, Acid maltase, Aglucosidase alfa, 76 kDa lysosomal alpha-glucosidase, 70 kDa lysosomal alpha-glucosidase, GAA
Calculated MW	105324
Application Details	Western blot, 0.1-0.5 µg/ml, Human Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Subcellular Localization	Lysosome
Source	Eukaryota
Protein Name	Lysosomal alpha-glucosidase
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names	Clone: 2G7
Immunogen	A synthetic peptide corresponding to a sequence in the middle region of human GAA, different from the related mouse sequence by eight amino acids, and from the related rat sequence by six amino acids.
Purification	Immunogen affinity purified.
Cross Reactivity	No cross-reactivity with other proteins.

Storage	Store at -20 °C for one year from date of receipt. After reconstitution, at 4 °C for one month. It can also be aliquotted and stored frozen at -20 °C for six months. Avoid repeated freeze-thaw cycles.
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Protein Information

Name	GAA
Function	Essential for the degradation of glycogen in lysosomes (PubMed: 14695532 , PubMed: 18429042 , PubMed: 1856189 , PubMed: 7717400). Has highest activity on alpha-1,4-linked glycosidic linkages, but can also hydrolyze alpha-1,6-linked glucans (PubMed: 29061980).
Cellular Location	Lysosome. Lysosome membrane

Background

Lysosomal alpha-glucosidase is an enzyme that in humans is encoded by the GAA gene. This gene encodes lysosomal alpha-glucosidase, which is essential for the degradation of glycogen to glucose in lysosomes. The encoded preproprotein is proteolytically processed to generate multiple intermediate forms and the mature form of the enzyme. Defects in this gene are the cause of glycogen storage disease II, also known as Pompe's disease, which is an autosomal recessive disorder with a broad clinical spectrum. Alternative splicing results in multiple transcript variants.

Images

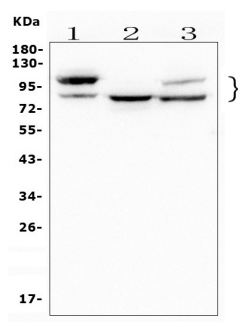


Figure 1. Western blot analysis of GAA using anti-GAA antibody (M01548).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human A549 tissue lysates,

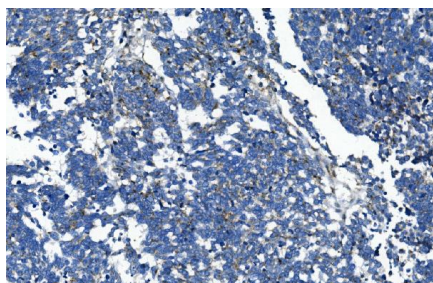
Lane 2: human HEK293 whole cell lysates,

Lane 3: human PC-3 whole cell lysates,

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-GAA antigen affinity purified polyclonal antibody (Catalog # M01548) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for GAA at approximately 110,95,76KD. The expected band size for GAA is at 105KD.

Figure 2. IHC analysis of GAA using anti-GAA antibody (M01548).

GAA was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with



10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-GAA Antibody (M01548) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

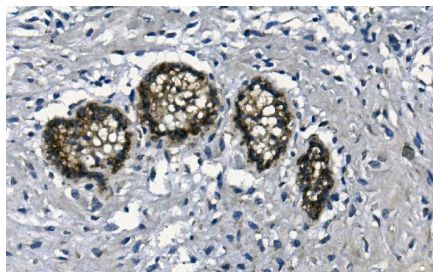


Figure 3. IHC analysis of GAA using anti-GAA antibody (M01548). GAA was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-GAA Antibody (M01548) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

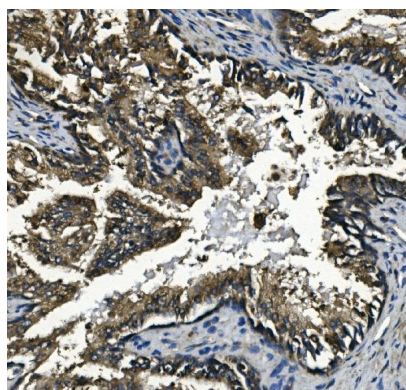


Figure 4. IHC analysis of GAA using anti-GAA antibody (M01548). GAA was detected in paraffin-embedded section of human prostatic cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-GAA Antibody (M01548) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

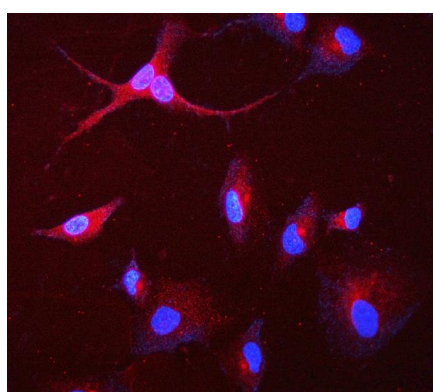
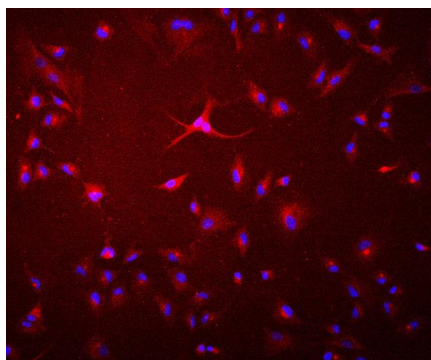


Figure 5. IF analysis of GAA using anti-GAA antibody (M01548). GAA was detected in immunocytochemical section of A549 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-GAA Antibody (M01548) overnight at 4°C. Cy3 Conjugated Goat Anti-Mouse IgG (BA1031) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Figure 6. IF analysis of GAA using anti-GAA antibody (M01548). GAA was detected in immunocytochemical section of A549 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-GAA Antibody (M01548) overnight at 4°C. Cy3 Conjugated Goat



Anti-Mouse IgG (BA1031) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

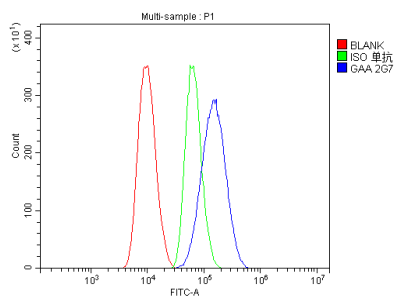


Figure 7. Flow Cytometry analysis of THP-1 cells using anti-GAA antibody M01548).

Overlay histogram showing THP-1 cells stained with M00377-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-GAA Antibody (M00377-2, 1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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