

Anti-Cytochrome C CYCS Antibody Picoband™ (monoclonal, 15F10)

Catalog # ABO14783

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P99999
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Cytochrome C CYCS Antibody Picoband™ (monoclonal, 15F10) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	54205
Other Names	Cytochrome c, CYCS, CYC
Calculated MW	11749
Application Details	Western blot, 0.1-0.5 µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells
Subcellular Localization	Mitochondrion intermembrane space. Loosely associated with the inner membrane.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names Immunogen	Clone: 15F10 E.coli-derived human Cytochrome C recombinant protein (Position: G2-E105). Human Cytochrome C shares 91% amino acid (aa) sequence identity with both mouse and rat Cytochrome C.
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	CYCS
Synonyms	CYC
Function	Electron carrier protein. The oxidized form of the cytochrome c heme group can accept an electron from the heme group of the cytochrome c1 subunit of cytochrome reductase. Cytochrome c then transfers this electron to the cytochrome oxidase complex, the final protein carrier in the mitochondrial electron-transport chain.
Cellular Location	Mitochondrion intermembrane space. Note=Loosely associated with the inner membrane

Background

CYCS is also known as CYC, HCS or THC4. This gene encodes a small heme protein that functions as a central component of the electron transport chain in mitochondria. The encoded protein associates with the inner membrane of the mitochondrion where it accepts electrons from cytochrome b and transfers them to the cytochrome oxidase complex. This protein is also involved in initiation of apoptosis. Mutations in this gene are associated with autosomal dominant nonsyndromic thrombocytopenia. Numerous processed pseudogenes of this gene are found throughout the human genome.

Images

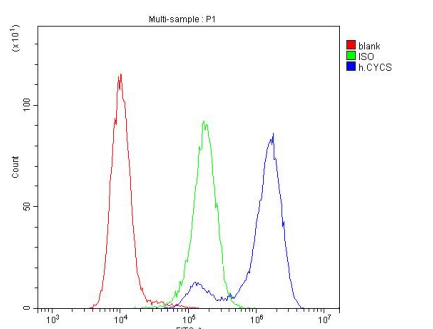


Figure 3. Flow Cytometry analysis of K562 cells using anti-Cytochrome C antibody (M03529-5). Overlay histogram showing K562 cells stained with M03529-5 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cytochrome C Antibody (M03529-5, 1 µg/1x10⁶ cells) 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.

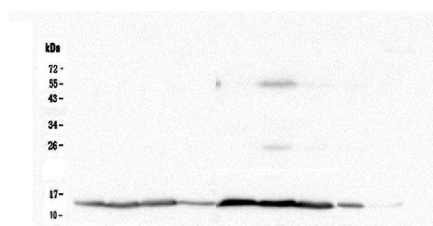


Figure 8. Western blot analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5). Electrophoresis was performed on a 12% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50µg of sample under reducing conditions. Lane 1: rat brain tissue lysate, Lane 2: rat heart tissue lysate, Lane 3: rat kidney tissue lysate, Lane 4: rat testis tissue lysate, Lane 5: mouse brain tissue lysate, Lane 6: mouse heart tissue lysate,

Lane 7: mouse kidney tissue lysate,
 Lane 8: mouse testis tissue lysate,
 Lane 9: mouse Neuro-2a whole cell lysate.
 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 rabbit anti-Cytochrome C antigen affinity purified polyclonal antibody (Catalog # M03529-5) 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-rabbit 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 # EK1002) with Tanon 5200 system.

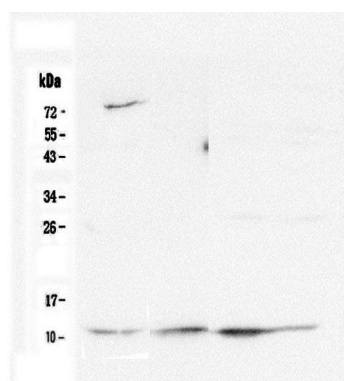


Figure 7. Western blot analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5). Electrophoresis was performed on a 12% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysate,
 Lane 2: human HepG2 whole cell lysate
 Lane 3: human K562 whole cell lysate,
 Lane 4: human Caco-2 whole cell lysate.

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-Cytochrome C antigen affinity purified monoclonal antibody (Catalog # M03529-5) 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.

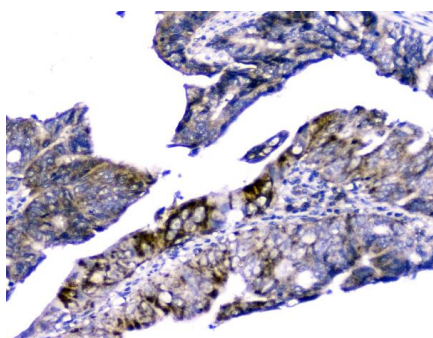
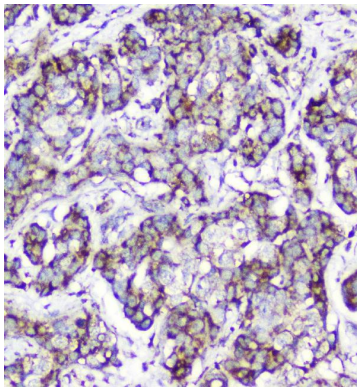


Figure 5. IHC analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5). Cytochrome C was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Cytochrome C Antibody (M03529-5) 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 6. IHC analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5). Cytochrome C was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6,



epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 $\mu\text{g}/\text{ml}$ mouse anti-Cytochrome C Antibody (M03529-5) 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.

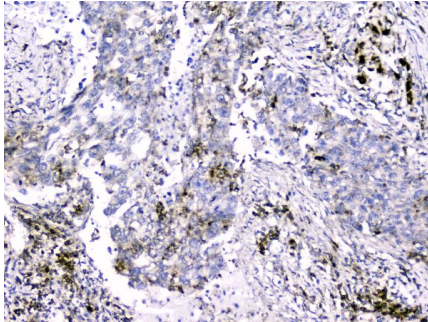


Figure 4. IHC analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5). Cytochrome C was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 $\mu\text{g}/\text{ml}$ mouse anti-Cytochrome C Antibody (M03529-5) 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.

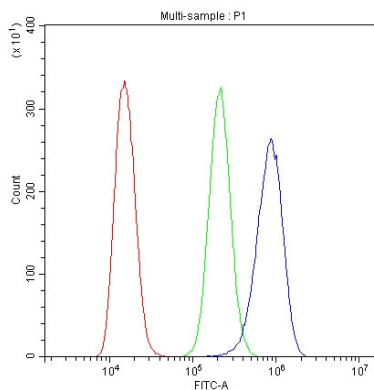


Figure 2. Flow Cytometry analysis of A431 cells using anti-Cytochrome C antibody (M03529-5). Overlay histogram showing A431 cells stained with M03529-5 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cytochrome C Antibody (M03529-5, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

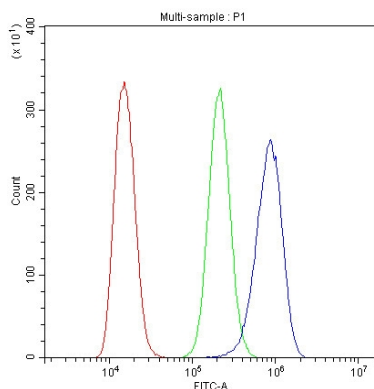
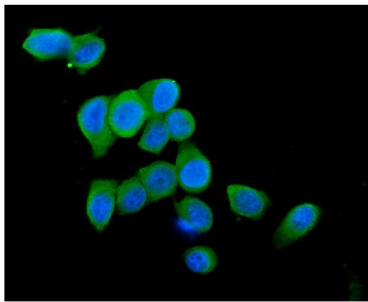


Figure 1. Flow Cytometry analysis of A431 cells using anti-Cytochrome C antibody (M03529-5). Overlay histogram showing A431 cells stained with M03529-5 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cytochrome C Antibody (M03529-5, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Figure 9. IF analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5). Cytochrome C was detected in immunocytochemical section of MCF7 cells. 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-Cytochrome C Antibody (M03529-5) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) 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.

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