10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

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

Catalog # ABO14783

Specification

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

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

Gene ID 54205

Other Names
Cytochrome c, CYCS, CYC

Calculated MW
14 kDa KDa

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/1x106 cells

Subcellular Localization
Mitochondrion intermembrane space. Loosely associated with the inner membrane.

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.

Immunogen
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.

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

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

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

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

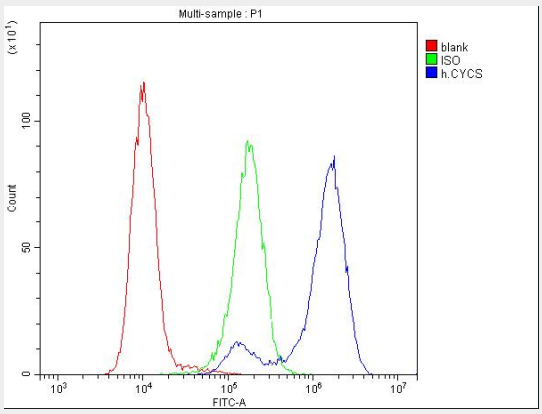

Figure 3. Flow Cytometry analysis of K562 cells using anti-Cytochrome C antibody (M03529-5). Overlay histogram showing K562 cells stained with M03529-5 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cytochrome C Antibody (M03529-5,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype

control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

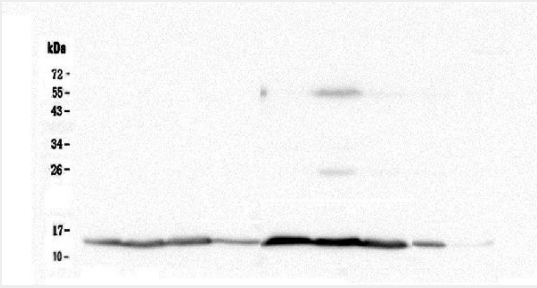

Figure 8. Western blot analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5). Electrophoresis was performed on a 12% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat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

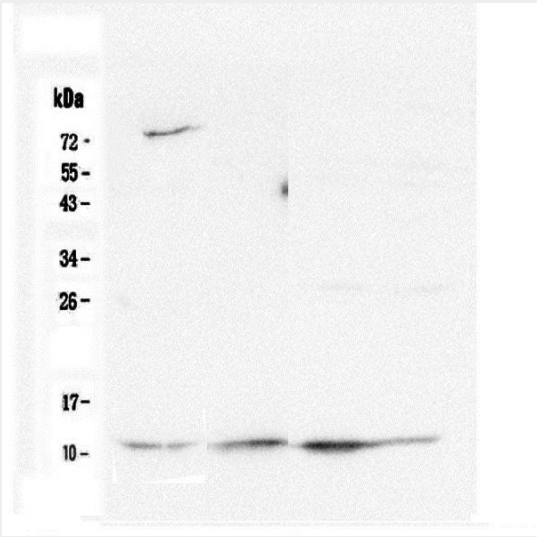

Figure 7. Western blot analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5). Electrophoresis was performed on a 12% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human 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.

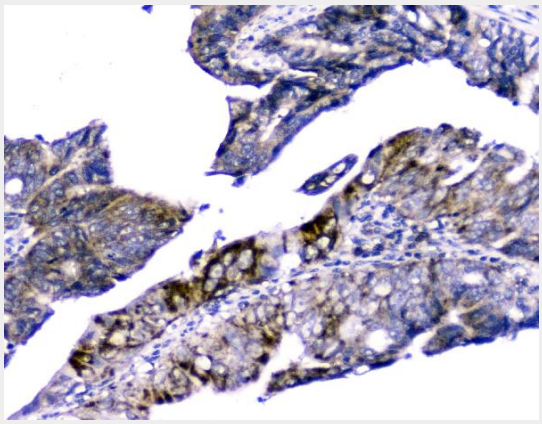

Figure 5. IHC analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5).
Cytochrome C was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

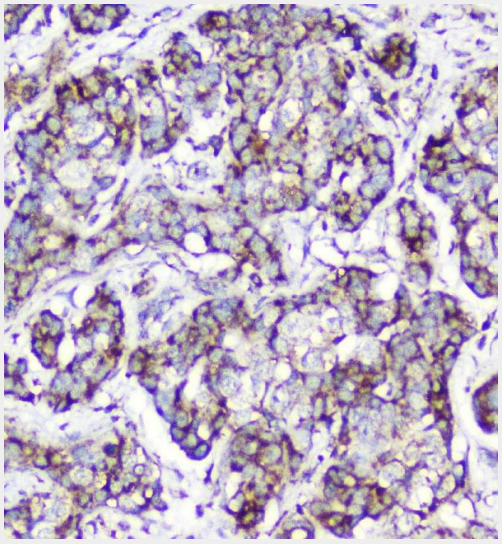

Figure 6. IHC analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5).
Cytochrome C was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Cytochrome C Antibody (M03529-5) overnight at 4°C.

10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

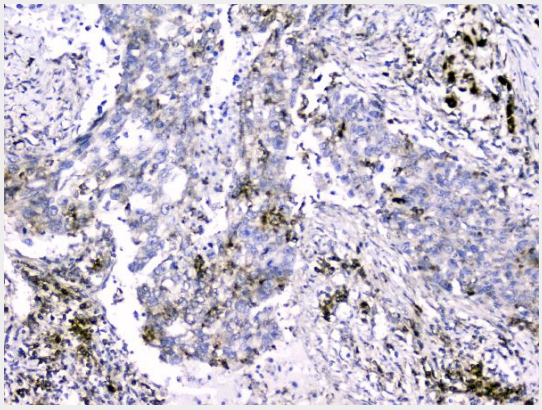

Figure 4. IHC analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5).
Cytochrome C was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 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 Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

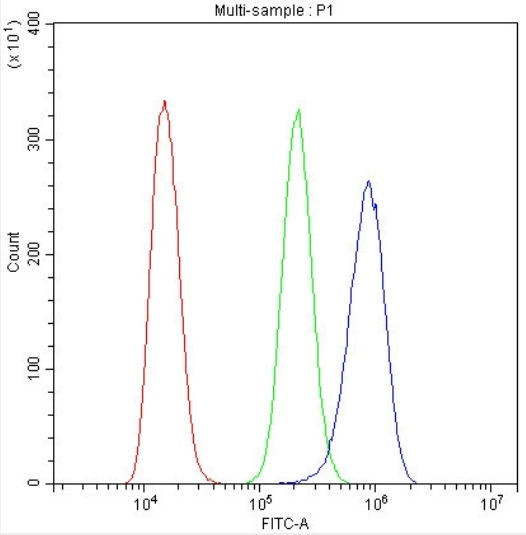

Figure 2. Flow Cytometry analysis of A431 cells using anti-Cytochrome C antibody (M03529-5).
Overlay histogram showing A431 cells stained with M03529-5 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cytochrome C Antibody (M03529-5,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

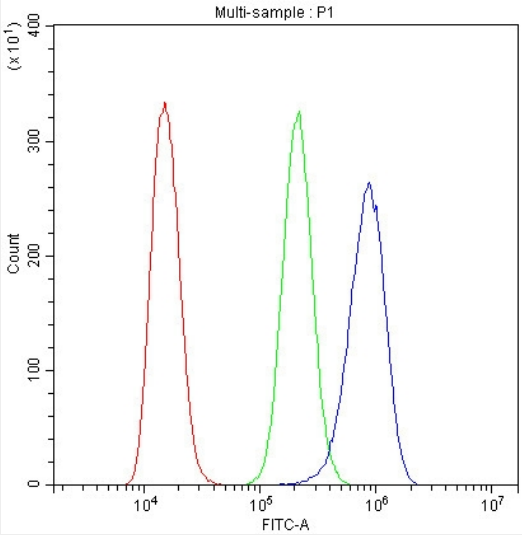

Figure 1. Flow Cytometry analysis of A431 cells using anti-Cytochrome C antibody (M03529-5). Overlay histogram showing A431 cells stained with M03529-5 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cytochrome C Antibody (M03529-5,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

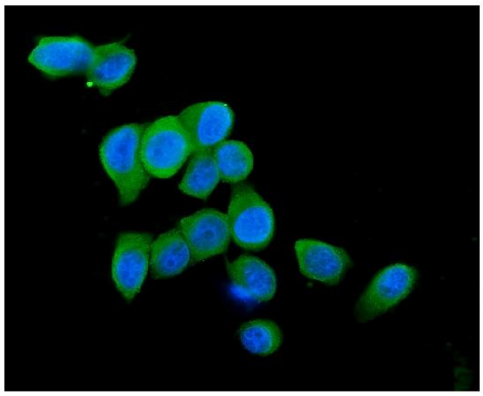

Figure 9. IF analysis of Cytochrome C using anti-Cytochrome C antibody (M03529-5). Cytochrome C was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. 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.

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