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Anti-CLPX Antibody Picoband™ (monoclonal, 11C11)

Catalog # ABO14896

Product Information

Application	WB, IF, ICC, FC
Primary Accession	O76031
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-CLPX Antibody Picoband™ (monoclonal, 11C11) . Tested in Flow Cytometry, IF, 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	10845
Other Names	ATP-dependent Clp protease ATP-binding subunit clpX-like, mitochondrial, CLPX
Calculated MW	69224
Application Details	Western blot, 0.1-0.5 μg/ml, Human Immunocytochemistry/Immunofluorescence, 2 μg/ml, Human Flow Cytometry, 1-3 μg/1x10^6 cells, Human
Subcellular Localization	Mitochondrion; mitochondrion nucleoid
Tissue Specificity	Higher expression in skeletal muscle and heart and to a lesser extent in liver, brain, placenta, lung, kidney and pancreas.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 11C11
Immunogen	E. coli-derived human CLPX recombinant protein (Position: A337-E574).
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.

Protein Information

Name	CLPX (HGNC:2088)
Function	ATP-dependent chaperone that functions as an unfoldase. As part of the ClpXP protease complex, it recognizes specific protein substrates, unfolds them using energy derived from ATP hydrolysis, and then translocates them to the proteolytic subunit (CLPP) of the ClpXP complex for degradation (PubMed:11923310, PubMed:22710082, PubMed:28874591). Thanks to its chaperone activity, it also functions in the incorporation of the pyridoxal phosphate cofactor into 5- aminolevulinate synthase, thereby activating 5-aminolevulinate (ALA) synthesis, the first step in heme biosynthesis (PubMed:28874591). This chaperone is also involved in the control of mtDNA nucleoid distribution, by regulating mitochondrial transcription factor A (TFAM) activity (PubMed:22841477).
Cellular Location	Mitochondrion. Mitochondrion matrix, mitochondrion nucleoid
Tissue Location	Higher expression in skeletal muscle and heart and to a lesser extent in liver, brain, placenta, lung, kidney and pancreas.

Background

ATP-dependent Clp protease ATP-binding subunit clpX-like, mitochondrial is an enzyme that in humans is encoded by the CLPX gene. This protein is a member of the family of AAA Proteins (AAA+ ATPase) and is to form the protein complex of Clp protease. The protein encoded by this gene is part of a protease found in mitochondria. This protease is ATP-dependent and targets specific proteins for degradation. The protease consists of two heptameric rings of the CLPP catalytic subunit sandwiched between two hexameric rings of the chaperone subunit encoded by this gene. Targeted proteins are unwound by this protein and then passed on to the CLPP subunit for degradation. Two transcript variants, one protein-coding and the other non-protein coding, have been found for this gene.

Images

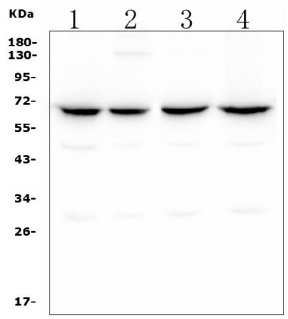


Figure 1. Western blot analysis of CLPX using anti ZO-1 antibody (M00978).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Raji tissue lysates,
Lane 2: human Hela whole cell lysates,
Lane 3: human HepG2 whole cell lysates,
Lane 4: human Caco-2 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-CLPX antigen affinity purified polyclonal antibody (Catalog # M00978) 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 CLPX at approximately 69KD. The expected band size for CLPX is at 69KD.

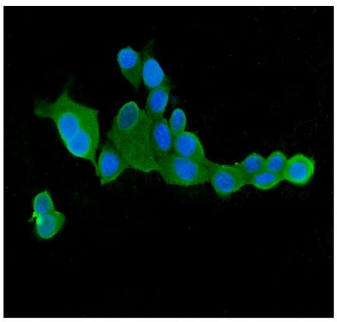


Figure 2. IF analysis of CLPX using anti-CLPX antibody (M00978).
CLPX was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-CLPX Antibody (M00978) 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.

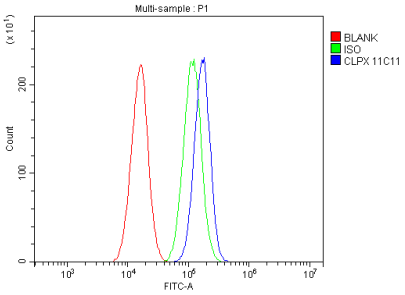


Figure 3. Flow Cytometry analysis of A549 cells using anti-CLPX antibody (M00978).
Overlay histogram showing A549 cells stained with M00978 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-CLPX Antibody (M00978,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 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.

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