

Anti-Cofilin 2/CFL2 Antibody Picoband™ (monoclonal, 8C13)

Catalog # ABO14866

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	Q9Y281
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Cofilin 2/CFL2 Antibody Picoband™ (monoclonal, 8C13) . 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 500 µg/ml.

Additional Information

Gene ID	1073
Other Names	Cofilin-2, Cofilin, muscle isoform, CFL2
Calculated MW	18737
Application Details	Western blot, 0.1-0.5 µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml Immunocytochemistry/Immunofluorescence, 2 µg/ml Flow Cytometry, 1-3 µg/1x10 ⁶ cells
Subcellular Localization	Cytoskeleton. Nucleus matrix.
Tissue Specificity	Isoform CFL2b is expressed predominantly in skeletal muscle and heart. Isoform CFL2a is expressed in various tissues.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names	Clone: 8C13
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human Cofilin 2/CFL2, identical to the related mouse sequence.
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	CFL2
Function	Controls reversibly actin polymerization and depolymerization in a pH-sensitive manner. Its F-actin depolymerization activity is regulated by association with CSPR3 (PubMed: 19752190). It has the ability to bind G- and F-actin in a 1:1 ratio of cofilin to actin. It is the major component of intranuclear and cytoplasmic actin rods. Required for muscle maintenance. May play a role during the exchange of alpha-actin forms during the early postnatal remodeling of the sarcomere (By similarity).
Cellular Location	Nucleus matrix. Cytoplasm, cytoskeleton. Note=Colocalizes with CSPR3 in the Z line of sarcomeres.
Tissue Location	Isoform CFL2b is expressed predominantly in skeletal muscle and heart. Isoform CFL2a is expressed in various tissues

Background

Cofilin 2 (muscle), also known as CFL2, is a protein which in humans is encoded by the CFL2 gene. It is mapped to 14q12. This gene encodes an intracellular protein that is involved in the regulation of actin-filament dynamics. And this protein is a major component of intranuclear and cytoplasmic actin rods. It can bind G- and F-actin in a 1:1 ratio of cofilin to actin, and it reversibly controls actin polymerization and depolymerization in a pH-dependent manner. Mutations in this gene cause nemaline myopathy type 7, a form of congenital myopathy. Alternative splicing results in multiple transcript variants.

Images

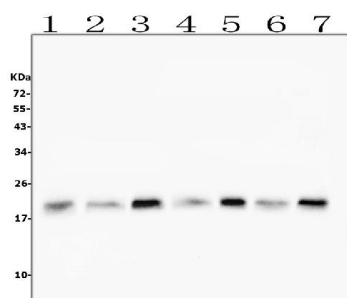


Figure 1. Western blot analysis of Cofilin-2 using anti-Cofilin-2 antibody (M04773).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates
Lane 2: human U2OS whole cell lysates
Lane 3: human HepG2 whole cell lysates
Lane 4: human T-47D whole cell lysates
Lane 5: human Raji whole cell lysates
Lane 6: human placenta tissue lysates
Lane 7: human A549 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-Cofilin-2 antigen affinity purified monoclonal antibody (Catalog # M04773) 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 Cofilin-2 at approximately 19KD. The expected band size for Cofilin-2 is at 19KD.

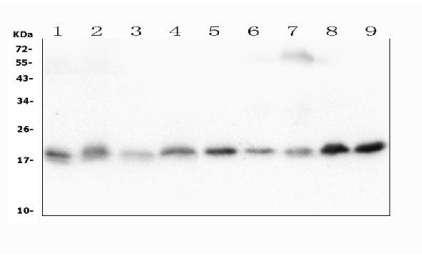


Figure 2. Western blot analysis of Cofilin-2 using anti-Cofilin-2 antibody (M04773).

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: rat heart tissue lysates

Lane 2: rat liver tissue lysates

Lane 3: rat kidney tissue lysates

Lane 4: rat brain tissue lysates

Lane 5: mouse heart tissue lysates

Lane 6: mouse liver tissue lysates

Lane 7: mouse kidney tissue lysates

Lane 8: mouse brain tissue lysates

Lane 9: mouse NIH/3T3 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-Cofilin-2 antigen affinity purified monoclonal antibody (Catalog # M04773) 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 Cofilin-2 at approximately 19KD. The expected band size for Cofilin-2 is at 19KD.

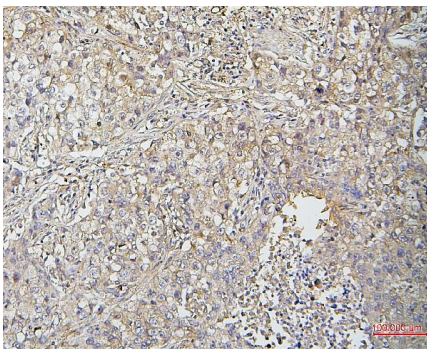


Figure 3. IHC analysis of Cofilin-2 using anti-Cofilin-2 antibody (M04773).

Cofilin-2 was detected in paraffin-embedded section of human lung cancer tissues. 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 1 µg/ml mouse anti-Cofilin-2 Antibody (M04773) 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.

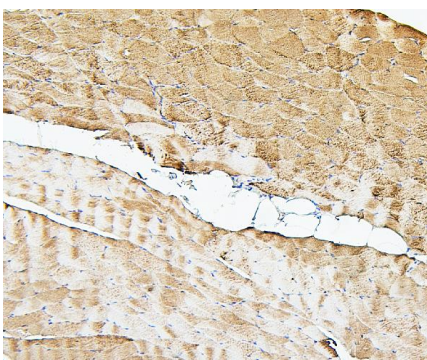


Figure 4. IHC analysis of Cofilin-2 using anti-Cofilin-2 antibody (M04773).

Cofilin-2 was detected in paraffin-embedded section of human skeletal muscle tissues. 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 1 µg/ml mouse anti-Cofilin-2 Antibody (M04773) 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.

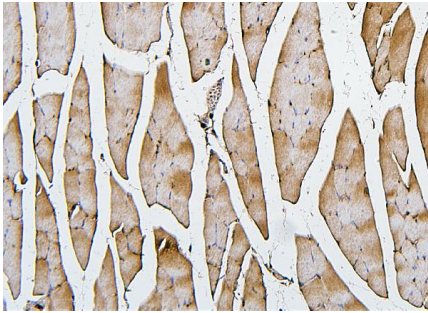


Figure 5. IHC analysis of Cofilin-2 using anti-Cofilin-2 antibody (M04773). Cofilin-2 was detected in paraffin-embedded section of mouse skeletal muscle tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Cofilin-2 Antibody (M04773) 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.

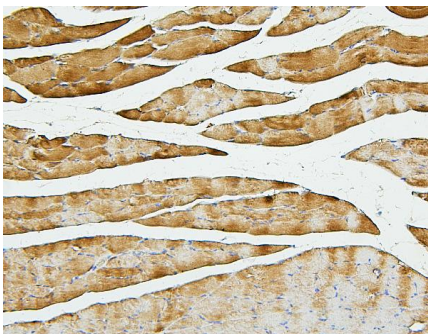


Figure 6. IHC analysis of Cofilin-2 using anti-Cofilin-2 antibody (M04773). Cofilin-2 was detected in paraffin-embedded section of rat skeletal muscle tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Cofilin-2 Antibody (M04773) 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.

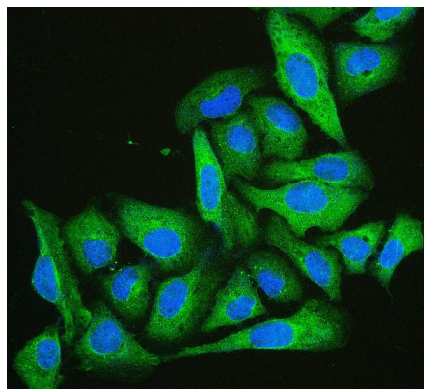


Figure 7. IF analysis of Cofilin-2 using anti-Cofilin-2 antibody (M04773). Cofilin-2 was detected in immunocytochemical section of U2OS cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-Cofilin-2 Antibody (M04773) 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.

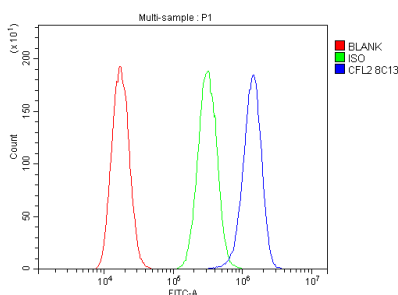


Figure 8. Flow Cytometry analysis of A549 cells using anti-Cofilin-2 antibody (M04773). Overlay histogram showing A549 cells stained with M04773 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cofilin-2 Antibody (M04773, 1 µg/1x10⁶ cells) for 30 min 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.

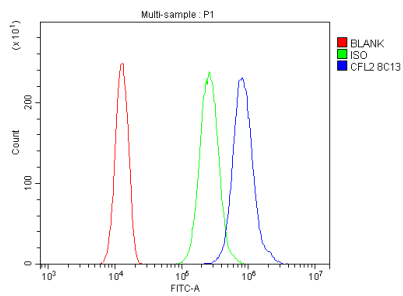


Figure 9. Flow Cytometry analysis of SiHa cells using anti-Cofilin-2 antibody (M04773). Overlay histogram showing SiHa cells stained with M04773 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cofilin-2 Antibody (M04773, $1 \mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C . DyLight®488 conjugated goat anti-mouse IgG (BA1126, $5\text{--}10 \mu\text{g}/1 \times 10^6$ 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.

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