

#### Anti-MCM5 Picoband<sup>™</sup> Antibody (monoclonal, 4G10) Catalog # ABO15010

Catalog # ABUISUI

# Anti-MCM5 Picoband<sup>™</sup> Antibody (monoclonal, 4G10) - Product Information

Application Primary Accession Host Isotype Reactivity Clonality Format Description WB, IHC, IF, ICC, FC <u>P33992</u> Mouse Mouse IgG2a Rat, Human, Mouse Monoclonal Lyophilized

Anti-MCM5 Picoband<sup>™</sup> Antibody (monoclonal, 4G10) . 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-MCM5 Picoband<sup>™</sup> Antibody (monoclonal, 4G10) - Additional Information

Gene ID 4174

Other Names DNA replication licensing factor MCM5 {ECO:0000303|Ref.3}, 3.6.4.12, CDC46 homolog, P1-CDC46, MCM5, CDC46

Calculated MW 95 kDa KDa

**Application Details** Western blot, 0.25-0.5 μg/ml, Human, Mouse, Rat<br> Immunohistochemistry (Paraffin-embedded Section), 2-5 μg/ml, Human<br> Immunocytochemistry/Immunofluorescence, 5 μg/ml, Human<br> Flow Cytometry, 1-3 μg/1x10^6 cells, Human<br>

**Contents** Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.

Immunogen E.coli-derived human MCM5 recombinant protein (Position: K206-H723).

**Purification** Immunogen affinity purified.

### 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-MCM5 Picoband<sup>™</sup> Antibody (monoclonal, 4G10) - Protein Information

Name MCM5

Synonyms CDC46

#### Function

Acts as a component of the MCM2-7 complex (MCM complex) which is the replicative helicase essential for 'once per cell cycle' DNA replication initiation and elongation in eukaryotic cells. Core component of CDC45-MCM-GINS (CMG) helicase, the molecular machine that unwinds template DNA during replication, and around which the replisome is built (PubMed:<a href="http://www.uniprot.org/citations/16899510" target="\_blank">16899510</a>, PubMed:<a href="http://www.uniprot.org/citations/32453425" target="\_blank">32453425</a>, PubMed:<a href="http://www.uniprot.org/citations/34694004" target="\_blank">34694004</a>, PubMed:<a href="http://www.uniprot.org/citations/34694004" target="\_blank">34694004</a>, PubMed:<a href="http://www.uniprot.org/citations/34694004" target="\_blank">34700328</a>, PubMed:<a href="http://www.uniprot.org/citations/34694004" target="\_blank">34700328</a>, PubMed:<a href="http://www.uniprot.org/citations/34700328" target="\_blank">35585232</a>). The active ATPase sites in the MCM2- 7 ring are formed through the interaction surfaces of two neighboring subunits such that a critical structure of a conserved arginine finger motif is provided in trans relative to the ATP-binding site of the Walker A box of the adjacent subunit. The six ATPase active sites, however, are likely to contribute differentially to the complex helicase activity (PubMed:<a href="http://www.uniprot.org/citations/32453425" target="\_blank">32453425</a>).

### **Cellular Location**

Nucleus. Chromosome. Note=Associated with chromatin before the formation of nuclei and detaches from it as DNA replication progresses.

## Anti-MCM5 Picoband<sup>™</sup> Antibody (monoclonal, 4G10) - Protocols

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

- <u>Western Blot</u>
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

### Anti-MCM5 Picoband<sup>™</sup> Antibody (monoclonal, 4G10) - Images



Figure 2. IHC analysis of MCM5 using anti-MCM5 antibody (M03642-2).

MCM5 was detected in paraffin-embedded section of human gallbladder adenocarcinoma 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 2  $\mu$ g/ml mouse anti-MCM5 Antibody (M03642-2) 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 3. IHC analysis of MCM5 using anti-MCM5 antibody (M03642-2).

MCM5 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 2  $\mu$ g/ml mouse anti-MCM5 Antibody (M03642-2) 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.

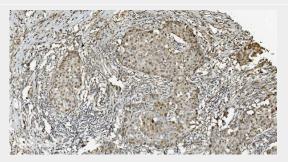


Figure 4. IHC analysis of MCM5 using anti-MCM5 antibody (M03642-2).

MCM5 was detected in paraffin-embedded section of human pancreatic 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 2  $\mu$ g/ml mouse anti-MCM5 Antibody (M03642-2) 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.

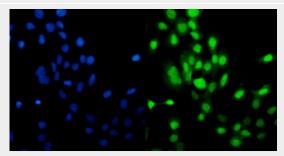


Figure 5. IF analysis of MCM5 using anti-MCM5 antibody (M03642-2).

MCM5 was detected in immunocytochemical section of A431 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 5  $\mu$ g/mL mouse anti-MCM5 Antibody (M03642-2) 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.

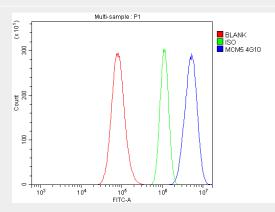


Figure 6. Flow Cytometry analysis of A549 cells using anti-MCM5 antibody (M03642-2). Overlay histogram showing A549 cells stained with M03642-2 (Blue line). The cells were blocked

with 10% normal goat serum. And then incubated with mouse anti-MCM5 Antibody (M03642-2, 1  $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10  $\mu$ g/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1  $\mu$ g/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

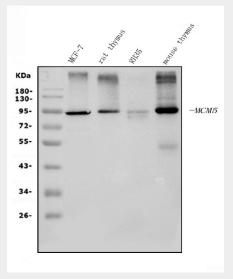


Figure 1. Western blot analysis of MCM5 using anti-MCM5 antibody (M03642-2).

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 MCF-7 whole cell lysates,

Lane 2: rat thymus tissue lysates,

Lane 3: rat RH-35 whole cell lysates,

Lane 4: mouse thymus tissue 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- MCM5 antigen affinity purified monoclonal antibody (Catalog # M03642-2) at 0.5  $\mu$ g/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 MCM5 at approximately 95KD. The expected band size for MCM5 is at 95KD.



## Anti-MCM5 Picoband<sup>™</sup> Antibody (monoclonal, 4G10) - Background

DNA replication licensing factor MCM5 is a protein that in humans is encoded by the MCM5 gene. It is mapped to 22q12.3. The protein encoded by this gene is structurally very similar to the CDC46 protein from S. cerevisiae, a protein involved in the initiation of DNA replication. The encoded protein is a member of the MCM family of chromatin-binding proteins and can interact with at least two other members of this family. The encoded protein is upregulated in the transition from the G0 to G1/S phase of the cell cycle and may actively participate in cell cycle regulation.