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Anti-DDX5 Antibody Picoband™ (monoclonal, 3F9)

Catalog # ABO14935

Specification

Anti-DDX5 Antibody Picoband™ (monoclonal, 3F9) - Product Information

Application	**WB, IHC, IHC-F, IF, ICC, FC**
Primary Accession	P17844
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-DDX5 Antibody Picoband™ (monoclonal, 3F9) . Tested in Flow Cytometry, IF, IHC, IHC-F, 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-DDX5 Antibody Picoband™ (monoclonal, 3F9) - Additional Information

Gene ID 1655

Other Names
Probable ATP-dependent RNA helicase DDX5, 3.6.4.13, DEAD box protein 5, RNA helicase p68, DDX5, G17P1, HELR, HLR1

Calculated MW
71 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Frozen Section), 0.5-1 µg/ml, Human
 Immunocytochemistry/Immunofluorescence, 2 µg/ml, Human
 Immunofluorescence, 2 µg/ml, Human, Mouse
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Subcellular Localization
Nucleus. Spliceosome.

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

Immunogen
E.coli-derived human DDX5 recombinant protein (Position: R85-K328).

Purification
Immunogen affinity purified.

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-DDX5 Antibody Picoband™ (monoclonal, 3F9) - Protein Information

Name DDX5

Synonyms G17P1, HELR, HLR1

Function
Involved in the alternative regulation of pre-mRNA splicing; its RNA helicase activity is necessary for increasing tau exon 10 inclusion and occurs in a RBM4-dependent manner. Binds to the tau pre- mRNA in the stem-loop region downstream of exon 10. The rate of ATP hydrolysis is highly stimulated by single-stranded RNA. Involved in transcriptional regulation; the function is independent of the RNA helicase activity. Transcriptional coactivator for androgen receptor AR but probably not ESR1. Synergizes with DDX17 and SRA1 RNA to activate MYOD1 transcriptional activity and involved in skeletal muscle differentiation. Transcriptional coactivator for p53/TP53 and involved in p53/TP53 transcriptional response to DNA damage and p53/TP53- dependent apoptosis. Transcriptional coactivator for RUNX2 and involved in regulation of osteoblast differentiation. Acts as a transcriptional repressor in a promoter-specific manner; the function probably involves association with histone deacetylases, such as HDAC1. As component of a large PER complex is involved in the inhibition of 3' transcriptional termination of circadian target genes such as PER1 and NR1D1 and the control of the circadian rhythms.

Cellular Location
Nucleus. Nucleus, nucleolus Nucleus speckle. Cytoplasm. Note=During the G0 phase, predominantly located in the nucleus. Cytoplasmic levels increase during the G1/S phase. During the M phase, located at the vicinity of the condensed chromosomes. At G1, localizes in the cytoplasm

Anti-DDX5 Antibody Picoband™ (monoclonal, 3F9) - 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-DDX5 Antibody Picoband™ (monoclonal, 3F9) - Images

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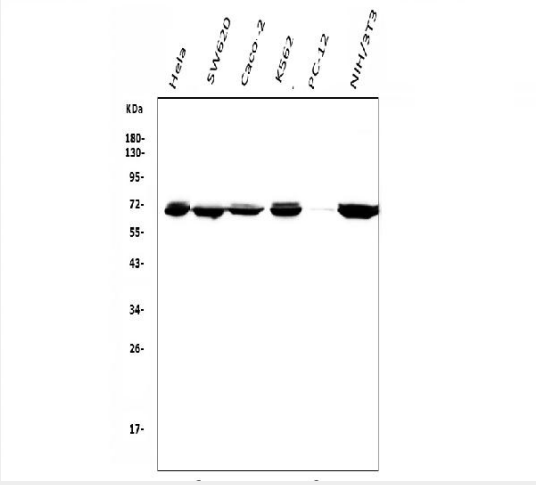

Figure 1. Western blot analysis of DDX5 using anti-DDX5 antibody (M00670-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates;
Lane 2: human SW620 whole cell lysates;
Lane 3: human Caco-2 whole cell lysates;
Lane 4: human K562 whole cell lysates;
Lane 5: rat PC-12 whole cell lysates;
Lane 6: 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-DDX5 antigen affinity purified monoclonal antibody (Catalog # M00670-1) 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 DDX5 at approximately 71KD. The expected band size for DDX5 is at 69KD.

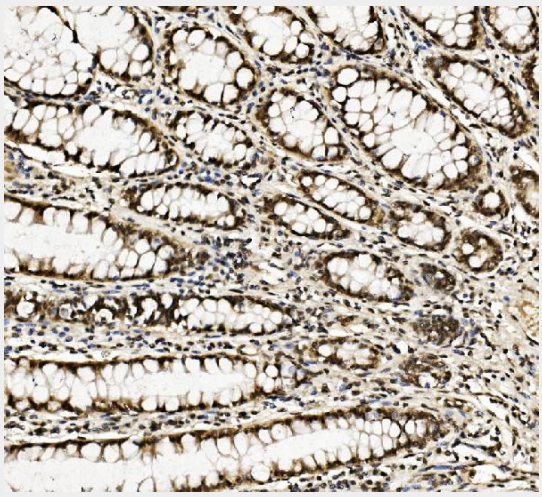

Figure 2. IHC analysis of DDX5 using anti-DDX5 antibody (M00670-1).
DDX5 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-DDX5 Antibody (M00670-1) 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.

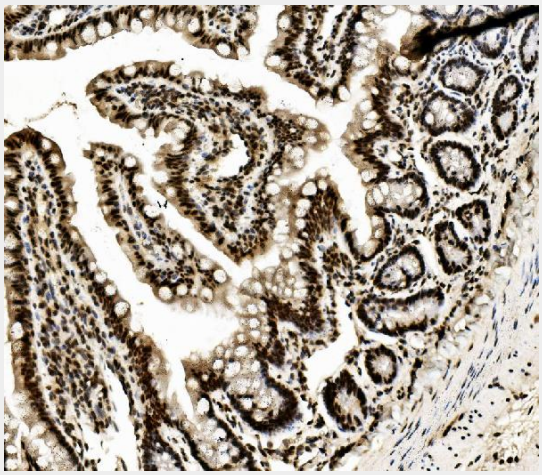

Figure 3. IHC analysis of DDX5 using anti-DDX5 antibody (M00670-1).
DDX5 was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-DDX5 Antibody (M00670-1) 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.

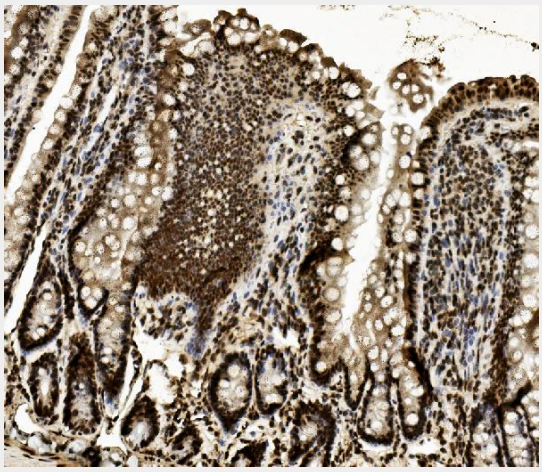

Figure 4. IHC analysis of DDX5 using anti-DDX5 antibody (M00670-1).
DDX5 was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-DDX5 Antibody (M00670-1) 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.

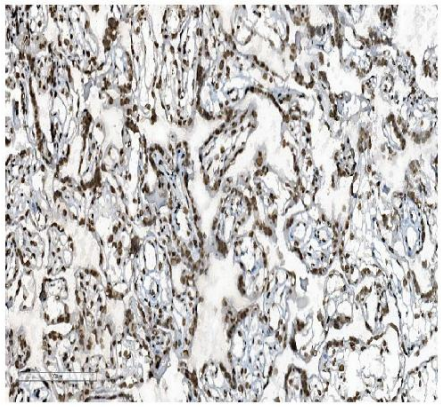

Figure 5. IHC analysis of DDX5 using anti-DDX5 antibody (M00670-1).
DDX5 was detected in frozen section of human placenta tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-DDX5 Antibody (M00670-1) overnight at 4°C. Biotinylated goat 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.

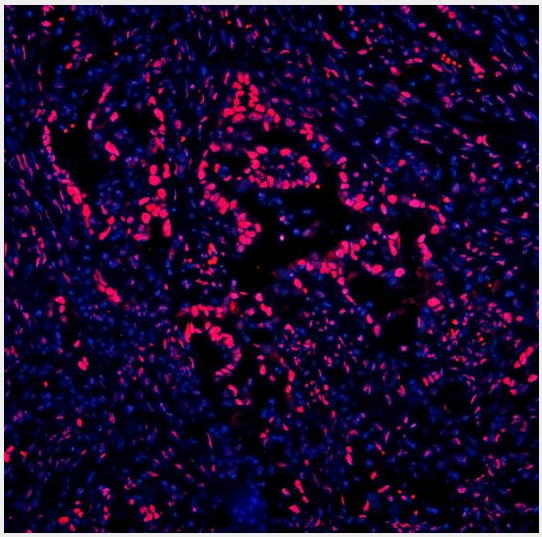

Figure 6. IF analysis of DDX5 using anti-DDX5 antibody (M00670-1).
DDX5 was detected in paraffin-embedded section of human intestine cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/mL mouse anti-DDX5 Antibody (M00670-1) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Mouse IgG (BA1141) 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.

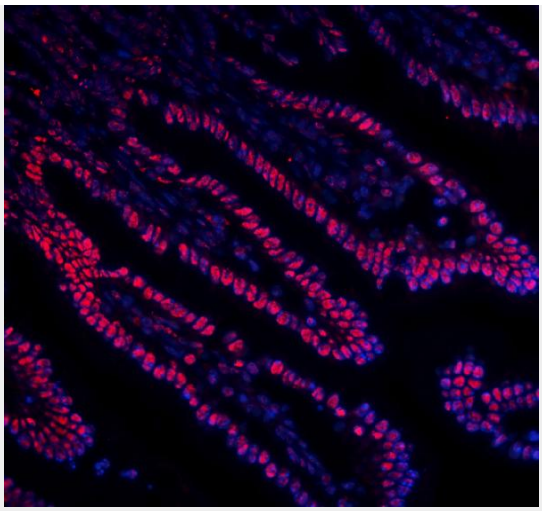

Figure 7. IF analysis of DDX5 using anti-DDX5 antibody (M00670-1).
DDX5 was detected in paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/mL mouse anti-DDX5 Antibody (M00670-1) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Mouse IgG (BA1141) 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.

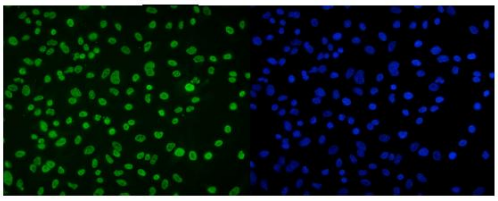

Figure 8. IF analysis of DDX5 using anti-DDX5 antibody (M00670-1).
DDX5 was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-DDX5 Antibody (M00670-1) 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.

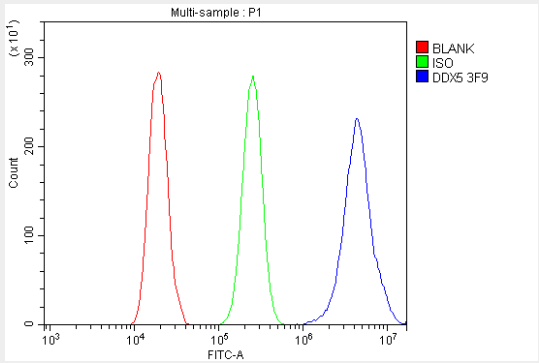

Figure 9. Flow Cytometry analysis of A431 cells using anti-DDX5 antibody (M00670-1).
Overlay histogram showing A431 cells stained with M00670-1 (Blue line).The cells were blocked

with 10% normal goat serum. And then incubated with mouse anti-DDX5 Antibody (M00670-1, 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.

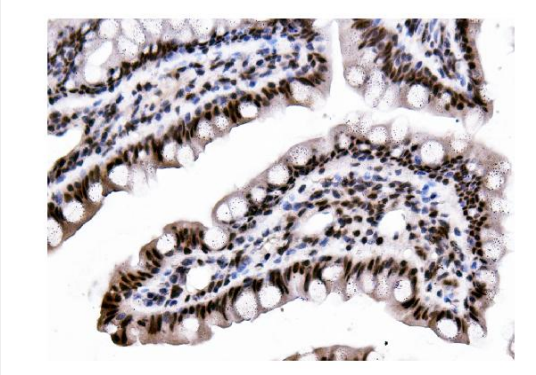

Figure 10. IHC analysis of Hexokinase 1/HK1 using anti-Hexokinase 1/HK1 antibody (M00670-1). Hexokinase 1/HK1 was detected in a paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Hexokinase 1/HK1 Antibody (M00670-1) 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.

Anti-DDX5 Antibody Picoband™ (monoclonal, 3F9) - Background

DDX5 (DEAD/H BOX 5), also known as HLR1 or G17P1, is an enzyme that in humans is encoded by the DDX5 gene. The p68 protein is a proliferation-associated nuclear antigen first identified through its highly specific cross-reaction with the simian virus 40 tumor antigen (Iggo et al., 1989). Subsequently, homology to eukaryotic translation initiation factor was found, and amino acid sequence blocks characteristic of a large superfamily of proteins with putative helicase activity were demonstrated. Brody et al. (1995) confirmed that this gene is located on chromosome 17 in the region of the BRCA1 gene at 17q21. By immunoprecipitation analysis, Caretti et al. (2006) found that p68, p72 (DDX17), and the noncoding RNA SRA (SRA1) associated with MYOD (MYOD1) in MYOD-transfected HeLa cells.