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

Anti-DDX6 Antibody Picoband™ (monoclonal, 8G6)

Catalog # ABO15065

Product Information

Application	WB, IF, ICC, FC
Primary Accession	P26196
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-DDX6 Antibody Picoband™ (monoclonal, 8G6) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	1656
Other Names	Probable ATP-dependent RNA helicase DDX6, 3.6.4.13, ATP-dependent RNA helicase p54, DEAD box protein 6, Oncogene RCK, DDX6, HLR2, RCK
Calculated MW	54417
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 8G6
Immunogen	E.coli-derived human DDX6 recombinant protein (Position: Q56-P483).
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.

Protein Information

Name	DDX6

Synonyms	HLR2, RCK
Function	Essential for the formation of P-bodies, cytosolic membrane- less ribonucleoprotein granules involved in RNA metabolism through the coordinated storage of mRNAs encoding regulatory functions (PubMed:25995375, PubMed:27342281, PubMed:31422817). Plays a role in P-bodies to coordinate the storage of translationally inactive mRNAs in the cytoplasm and prevent their degradation (PubMed:27342281). In the process of mRNA degradation, plays a role in mRNA decapping (PubMed:16364915). Blocks autophagy in nutrient-rich conditions by repressing the expression of ATG-related genes through degradation of their transcripts (PubMed:26098573).
Cellular Location	Cytoplasm, P-body. Cytoplasm. Nucleus. Cytoplasm, Cytoplasmic ribonucleoprotein granule {ECO:0000250|UniProtKB:P54823}. Note=Imported in the nucleus via interaction with EIF4ENIF1/4E-T via a piggy-back mechanism (PubMed:28216671). Upon cellular stress, relocalizes to stress granules (PubMed:26184334).
Tissue Location	Abundantly expressed in most tissues.

Background

DDX6 (DEAD/H BOX 6), also known as HLR2 or p54, is an enzyme that in humans is encoded by the DDX6 gene. DDX6 belongs to the DEAD box family of putative RNA helicases that contain a characteristic asp-glu-ala-asp (DEAD) box motif (Seto et al., 1995). Tunnacliffe et al. (1993) assigned the DDX6 gene more precisely using a panel of sequence tagged sites (STSs) representing 30 markers previously assigned to 11q23. Using mass spectroscopy, Fenger-Gron et al. (2005) found that RCK, EDC3 (YJDC), and HEDLS (RCD8) coimmunopurified with DCP1A and DCP2 from HEK293 cell lysates. Overexpression of DCP2, RCK, or EDC3 in HeLa cells reduced the association of endogenous DCP1A and XRN1 with cytoplasmic P bodies.

Images

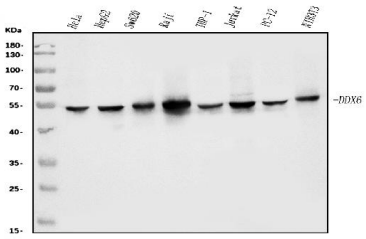


Figure 1. Western blot analysis of DDX6 using anti-DDX6 antibody (M03826-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human HepG2 whole cell lysates,
Lane 3: human Sw620 whole cell lysates,
Lane 4: human Raji whole cell lysates,
Lane 5: human THP-1 whole cell lysates,
Lane 6: human Jurkat whole cell lysates,
Lane 7: rat PC-12 whole cell lysates,
Lane 8: 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-DDX6 antigen affinity purified monoclonal antibody (Catalog # M03826-1) 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 DDX6 at approximately 54KD. The expected band size for DDX6 is at 54KD.

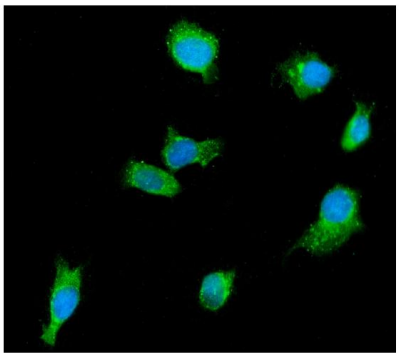


Figure 2. IF analysis of DDX6 using anti-DDX6 antibody (M03826-1).
DDX6 was detected in immunocytochemical section of MCF-7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-DDX6 Antibody (M03826-1) 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.

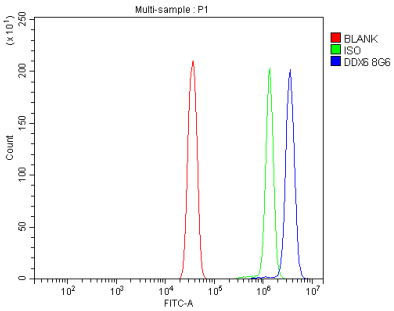


Figure 3. Flow Cytometry analysis of Hela cells using anti-DDX6 antibody (M03826-1).
Overlay histogram showing Hela cells stained with M03826-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-DDX6 Antibody (M03826-1, 1 µg/1x10^6 cells) for 30 min at 20°C. 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.

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