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

Anti-Ku70 Antibody Picoband™ (monoclonal, 9B6)

Catalog # ABO15042

Specification

Anti-Ku70 Antibody Picoband™ (monoclonal, 9B6) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P12956
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Human**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-Ku70 Antibody Picoband™ (monoclonal, 9B6) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Anti-Ku70 Antibody Picoband™ (monoclonal, 9B6) - Additional Information

Gene ID 2547

Other Names
X-ray repair cross-complementing protein 6, 3.6.4.-, 4.2.99.-, 5'-deoxyribose-5-phosphate lyase Ku70, 5'-dRP lyase Ku70, 70 kDa subunit of Ku antigen, ATP-dependent DNA helicase 2 subunit 1, ATP-dependent DNA helicase II 70 kDa subunit, CTC box-binding factor 75 kDa subunit, CTC75, CTCBF, DNA repair protein XRCC6, Lupus Ku autoantigen protein p70, Ku70, Thyroid-lupus autoantigen, TLAA, X-ray repair complementing defective repair in Chinese hamster cells 6, XRCC6, G22P1

Calculated MW
70 kDa KDa

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

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

Immunogen
A synthetic peptide corresponding to a sequence at C-terminus of human Ku70, different from the related mouse sequence by one amino acid.

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-Ku70 Antibody Picoband™ (monoclonal, 9B6) - Protein Information

Name XRCC6

Synonyms G22P1

Function
Single-stranded DNA-dependent ATP-dependent helicase that plays a key role in DNA non-homologous end joining (NHEJ) by recruiting DNA-PK to DNA (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). Required for double-strand break repair and V(D)J recombination (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). Also has a role in chromosome translocation (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). Has a role in chromosome translocation (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). The DNA helicase II complex binds preferentially to fork-like ends of double-stranded DNA in a cell cycle-dependent manner (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). It works in the 3'-5' direction (PubMed:11493912, PubMed:12145306, PubMed:<a

href="http://www.uniprot.org/citations/20493174" target="_blank">20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). During NHEJ, the XRCC5-XRRC6 dimer performs the recognition step: it recognizes and binds to the broken ends of the DNA and protects them from further resection (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). Binding to DNA may be mediated by XRCC6 (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). The XRCC5-XRRC6 dimer acts as a regulatory subunit of the DNA-dependent protein kinase complex DNA-PK by increasing the affinity of the catalytic subunit PRKDC to DNA by 100-fold (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). The XRCC5-XRRC6 dimer is probably involved in stabilizing broken DNA ends and bringing them together (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). The assembly of the DNA-PK complex to DNA ends is required for the NHEJ ligation step (PubMed:11493912, PubMed:12145306, PubMed:20493174, PubMed:2466842, PubMed:7957065, PubMed:8621488, PubMed:9742108). Probably also acts as a 5'-deoxyribose-5-phosphate lyase (5'-dRP lyase), by catalyzing the beta-elimination of the 5' deoxyribose-5-phosphate at an abasic site near double-strand breaks (PubMed:20383123). 5'-dRP lyase activity allows to 'clean' the termini of abasic sites, a class of nucleotide damage commonly associated with strand breaks, before such broken ends can be joined (PubMed:20383123). The XRCC5-XRRC6 dimer together with APEX1 acts as a negative regulator of transcription (PubMed:8621488). In association with NAA15, the XRCC5-XRRC6 dimer binds to the osteocalcin promoter and activates osteocalcin expression (PubMed:<a href="http://www.uniprot.org/citations/12145306"

target="_blank">12145306). Plays a role in the regulation of DNA virus-mediated innate immune response by assembling into the HDP-RNP complex, a complex that serves as a platform for IRF3 phosphorylation and subsequent innate immune response activation through the cGAS-STING pathway (PubMed:28712728). Negatively regulates apoptosis by interacting with BAX and sequestering it from the mitochondria (PubMed:15023334). Might have deubiquitination activity, acting on BAX (PubMed:18362350).

Cellular Location
Nucleus. Chromosome. Cytoplasm. Note=When trimethylated, localizes in the cytoplasm.

Anti-Ku70 Antibody Picoband™ (monoclonal, 9B6) - 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-Ku70 Antibody Picoband™ (monoclonal, 9B6) - Images

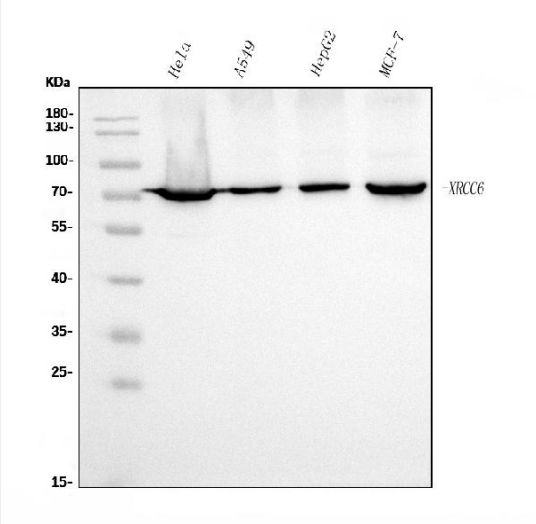

Figure 1. Western blot analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human A549 whole cell lysates,
Lane 3: human HepG2 whole cell lysates,
Lane 4: human MCF-7 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-Ku70 antigen affinity purified monoclonal antibody (Catalog # M01732-3) 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 Ku70 at approximately 70KD. The expected band size for Ku70 is at 70KD.

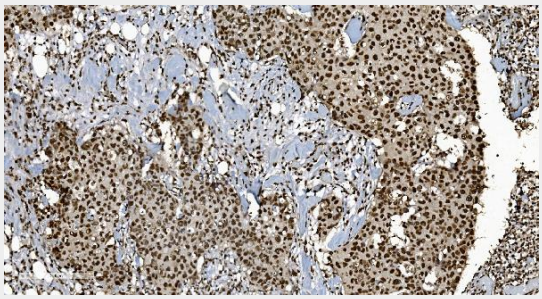

Figure 2. IHC analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ku70 Antibody (M01732-3) 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.

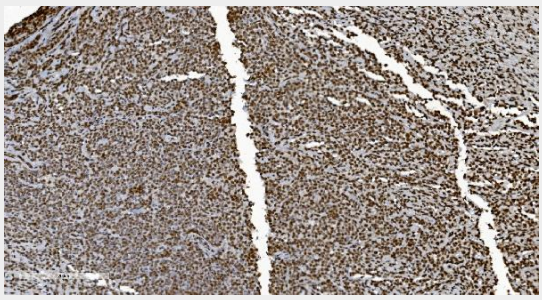

Figure 3. IHC analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in paraffin-embedded section of human lymphoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ku70 Antibody (M01732-3) 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.

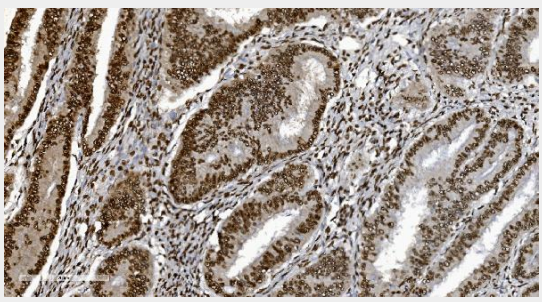

Figure 4. IHC analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated

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 2 µg/ml mouse anti-Ku70 Antibody (M01732-3) 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.

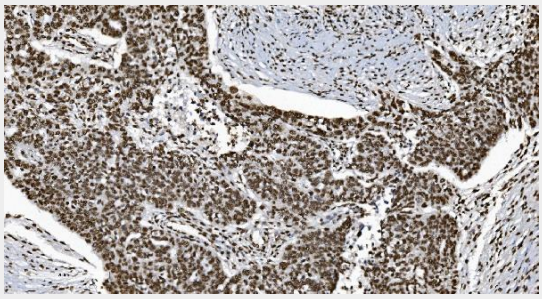

Figure 5. IHC analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in paraffin-embedded section of human ovarian serous adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ku70 Antibody (M01732-3) 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.

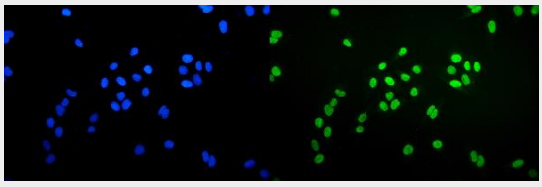

Figure 6. IF analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-Ku70 Antibody (M01732-3) 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.

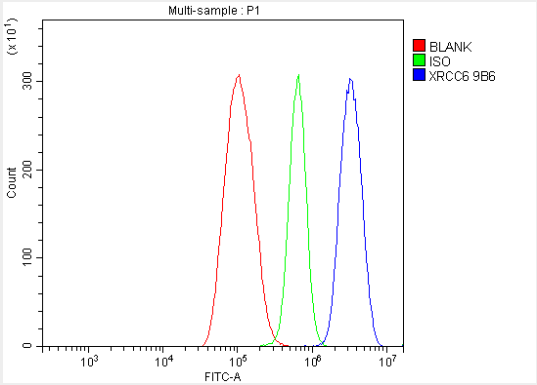

Figure 7. Flow Cytometry analysis of THP-1 cells using anti-Ku70 antibody (M01732-3).
Overlay histogram showing THP-1 cells stained with M01732-3 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Ku70 Antibody (M01732-3, 1

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.

Anti-Ku70 Antibody Picoband™ (monoclonal, 9B6) - Background

XRCC6 (X-Ray Repair, Complementing Defective, In Chinese Hamster, 6), also called Ku70, G22P1 or TLAA, is a protein that in humans, is encoded by the XRCC6 gene. In addition, the XRCC6 gene encodes subunit p70 of the p70/p80 autoantigen which consists of 2 proteins of molecular mass of approximately 70,000 and 80,000 daltons that dimerize to form a 10 S DNA-binding complex. The XRCC6 gene is mapped to 22q13.2. XRCC6 and Mre11 are differentially expressed during meiosis. XRCC6 interacts with Baxa, a mediator of mitochondrial-dependent apoptosis. Disruption of both FANCC and XRCC6 suppressed sensitivity to crosslinking agents, diminished chromosome breaks, and reversed defective homologous recombination. Ku70 binds directly to free DNA ends, committing them to NHEJ repair. In early meiotic prophase, however, when meiotic recombination is most probably initiated, Mre11 was abundant, whereas XRCC6 was not detectable.