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

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.

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	69843
Application Details	Western blot, 0.25-0.5 □g/ml, Human Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human 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: 9B6
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.

Protein Information

Name	XRCC6 (HGNC:4055)
Synonyms	G22P1
Function	DNA-binding protein critical for the DNA damage response, specifically in repairing double-strand breaks (DSBs) via the classical non-homologous end joining (NHEJ) pathway. It forms a heterodimer with XRCC5 (Ku80), creating the Ku70:Ku80 heterodimer (Ku complex), which serves as a DNA end-binding complex. It primarily binds DSBs and recruits essential repair factors, assembling the core long-range NHEJ complex to facilitate the alignment and ligation of broken DNA ends (PubMed:11493912, PubMed:20493174, PubMed:33854234, PubMed:34352203, PubMed:9742108). This pathway ensures the rapid repair of cytotoxic and mutagenic DSBs and contributes to the generation of diversity in T-cell receptors and antibodies through mechanisms such as V(D)J recombination (PubMed:9742108). Likely acts as a 5'-deoxyribose-5-phosphate lyase (5'-dRP lyase), catalyzing the beta-elimination of the 5'-deoxyribose- 5-phosphate at abasic sites near DSBs. This activity cleans the termini of abasic sites, a common form of nucleotide damage, preparing broken ends for ligation (PubMed:20383123). It may also possess 3'-5' DNA helicase activity, although this has not been confirmed in vivo, and its physiological significance remains unclear (PubMed:7957065). Beyond DNA repair, the protein contributes to telomere maintenance (PubMed:29490055). It is also implicated in transcriptional regulation, acting as a cofactor for various transcription factors (PubMed:12145306, PubMed:8621488). It 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). Can also bind RNAs and recruits PRKDC to a wide range of cellular RNAs, including the U3 small nucleolar RNA, playing a role in the biogenesis of ribosomal RNAs (PubMed:32103174). Additionally, it negatively regulates apoptosis by interacting with BAX, sequestering it from the mitochondria, and may possess deubiquitination activity targeting BAX (PubMed:15023334, PubMed:18362350, PubMed:35545041).
Cellular Location	Nucleus. Chromosome. Cytoplasm. Note=When trimethylated, localizes in the cytoplasm.

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.

Images

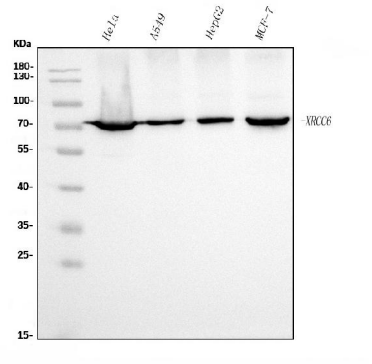


Figure 1. Western blot analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human Hela whole 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.

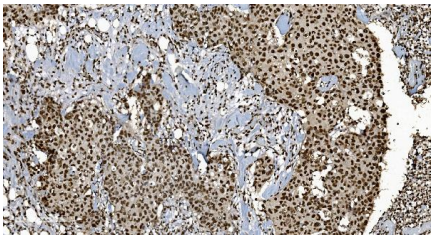


Figure 2. IHC analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue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

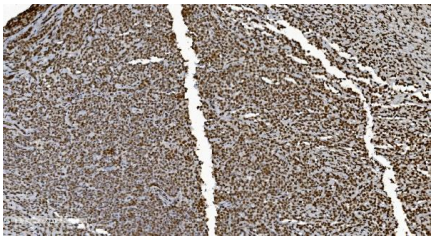


Figure 3. IHC analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in paraffin-embedded section of human lymphoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue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

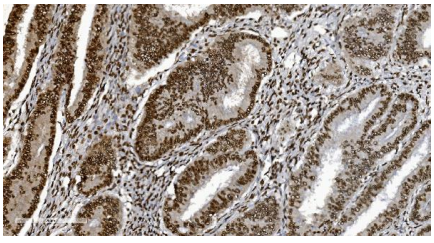


Figure 4. IHC analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with

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.

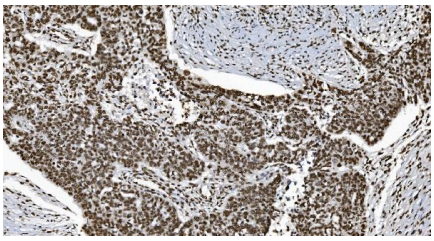


Figure 5. IHC analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in paraffin-embedded section of human ovarian serous adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat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

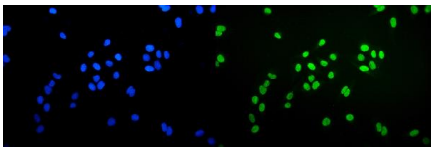


Figure 6. IF analysis of Ku70 using anti-Ku70 antibody (M01732-3).
Ku70 was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with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

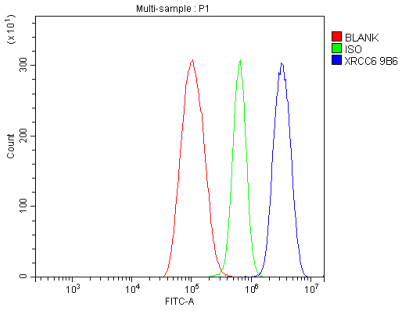


Figure 7. Flow Cytometry analysis of THP-1 cells using anti-Ku70 antibody (M01732-3).
Overlay histogram showing THP-1 cells stained with M01732-3 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Ku70 Antibody (M01732-3, 1 µg/1x10^6 cells) for 30 min at 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.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.