

## Basic Information

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|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| Product Name       | Anti-KU70/XRCC6 Antibody                                                                                                                                                                                                                                                                                                                                                                                                                    |  |
| Gene Name          | XRCC6                                                                                                                                                                                                                                                                                                                                                                                                                                       |  |
| Source             | Rabbit                                                                                                                                                                                                                                                                                                                                                                                                                                      |  |
| Isotype            | IgG                                                                                                                                                                                                                                                                                                                                                                                                                                         |  |
| Species Reactivity | human                                                                                                                                                                                                                                                                                                                                                                                                                                       |  |
| Tested Application | WB, IHC, ICC/IF, FCM                                                                                                                                                                                                                                                                                                                                                                                                                        |  |
| Contents           | 500 ug/ml antibody with PBS , 0.02% NaN <sub>3</sub> , 1 mg BSA and 50% glycerol.                                                                                                                                                                                                                                                                                                                                                           |  |
| Immunogen          | A synthetic peptide corresponding to a sequence at C-terminus of human Ku70 (464-496aa AIVEKLRFTYRSDSFENPVLQQHFRNLEALALD), different from the related mouse sequence by one amino acid.                                                                                                                                                                                                                                                     |  |
| concentration      | 500 ug/ml                                                                                                                                                                                                                                                                                                                                                                                                                                   |  |
| Purification       | Immunogen affinity purified.                                                                                                                                                                                                                                                                                                                                                                                                                |  |
| Observed MW        | 70KD                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |
| Dilution Ratios    | Western blot(WB): 1:500-2000<br>Immunohistochemistry in paraffin section (IHC): 1:50-400<br>Immunocytochemistry/Immunofluorescence (ICC/IF): 1:50-400<br>Flow cytometry (FCM): 1-3 µg/1x10 <sup>6</sup> cells<br>(Boiling the paraffin sections in 10mM citrate buffer,pH6.0,or PH8.0 EDTA repair liquid for 27 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user. |  |

## Storage

12 months from date of receipt, -20°C as supplied. 6 months 2 to 8°C after reconstitution. Avoid repeated freezing and thawing.

## Background Information

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.

## Selected Validation Data



Figure 1. Western blot analysis of Ku70 using anti-Ku70 antibody (PB9520). 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: A549 Whole Cell Lysate Lane 2: HELA Whole Cell Lysate Lane 3: HEPG2 Whole Cell Lysate Lane 4: MCF-7 Whole Cell Lysate 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 rabbit anti-Ku70 antigen affinity purified polyclonal antibody (Catalog # PB9520) 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-rabbit 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 # EK1002) with Tanon 5200 system. A specific band was detected for Ku70 at approximately 70KD. The expected band size for Ku70 is at 70KD.

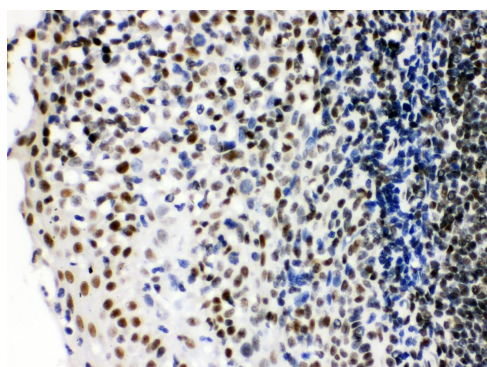


Figure 2. IHC analysis of Ku70 using anti-Ku70 antibody (PB9520). Ku70 was detected in paraffin-embedded section of Human Tonsil Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1µg/ml rabbit anti-Ku70 Antibody (PB9520) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

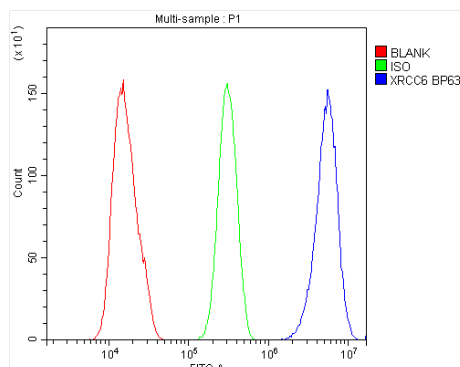


Figure 3. Flow Cytometry analysis of SiHa cells using anti- Ku70 antibody (PB9520).

Overlay histogram showing SiHa cells stained with PB9520 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Ku70 Antibody (PB9520,  $1\mu\text{g}/1\times 10^6$  cells) for 30 min at  $20^{\circ}\text{C}$ . DyLight488 conjugated goat anti-rabbit IgG (BA1127,  $5-10\mu\text{g}/1\times 10^6$  cells) was used as secondary antibody for 30 minutes at  $20^{\circ}\text{C}$ . Isotype control antibody (Green line) was rabbit IgG ( $1\mu\text{g}/1\times 10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

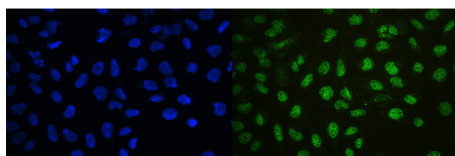


Figure 4. ICC analysis using anti- Ku70 antibody (PB9520). was detected in immersion fixed U2OS cell line. Cells were stained using the DyLight488-conjugated Anti-rabbit IgG Secondary Antibody (green)(Catalog # BA1127) and counterstained with DAPI (blue).