

Anti-Ku70/XRCC6 Antibody

Catalog Number: PA1642

About XRCC6

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.

Overview

Product Name	Anti-Ku70/XRCC6 Antibody
Reactive Species	Human
Description	Boster Bio Anti-Ku70/XRCC6 Antibody catalog # PA1642. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg Thimerosal, 0.05mg NaN3.
Storage Instructions	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.
Host	Rabbit
Uniprot ID	P12956

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human Ku70.
Predicted Reactive Species	Bovine
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized

Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml, Human</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, By Heat</p> <p>Immunocytochemistry , 0.5-1ug/ml, Human, -</p> <p>Immunocytochemistry/Immunofluorescence, 5ug/ml, Human</p> <p>Flow Cytometry, 1-3ug/1x10⁶ cells, Human</p>

Anti-Ku70/XRCC6 Antibody (PA1642) Images

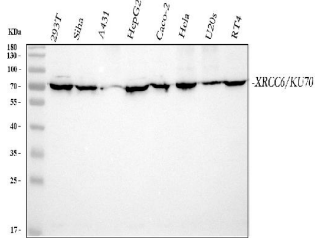


Figure 1. Western blot analysis of XRCC6 using anti-XRCC6 antibody (PA1642).

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 30 ug of sample under reducing conditions.

Lane 1: human 293T whole cell lysates,
Lane 2: human SiHa whole cell lysates,
Lane 3: human A431 whole cell lysates,
Lane 4: human HepG2 whole cell lysates,
Lane 5: human CACO-2 whole cell lysates,
Lane 6: human Hela whole cell lysates,
Lane 7: human U2OS whole cell lysates,
Lane 8: human RT4 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-XRCC6 antigen affinity purified polyclonal antibody (Catalog # PA1642) at 0.5 ug/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 1:5000 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 XRCC6 at approximately 70 kDa. The expected band size for XRCC6 is at 70 kDa.

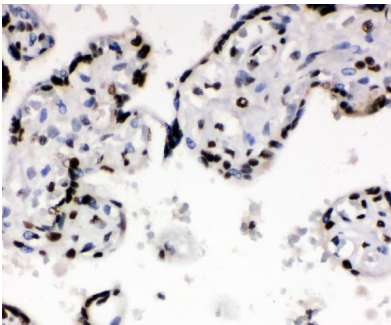


Figure 2. IHC analysis of XRCC6 using anti-XRCC6 antibody (PA1642).

XRCC6 was detected in a paraffin-embedded section of Human Placenta 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 ug/ml rabbit anti-XRCC6 Antibody (PA1642) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

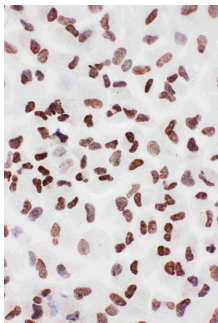


Figure 3. ICC analysis of XRCC6 using anti-XRCC6 antibody (PA1642).

XRCC6 was detected in an 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 1 ug/ml rabbit anti-XRCC6 Antibody (PA1642) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the

chromogen.

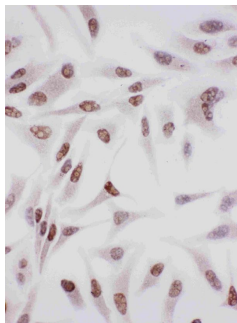


Figure 4. ICC analysis of XRCC6 using anti-XRCC6 antibody (PA1642). XRCC6 was detected in an immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 1 ug/ml rabbit anti-XRCC6 Antibody (PA1642) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

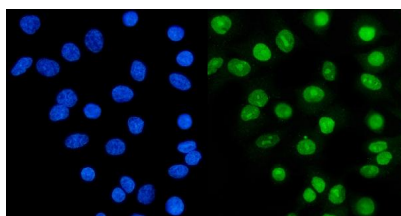


Figure 5. IF analysis of XRCC6 using anti-XRCC6 antibody (PA1642). XRCC6 was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL rabbit anti-XRCC6 Antibody (PA1642) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) 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.

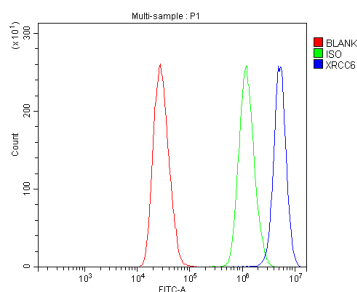


Figure 6. Flow Cytometry analysis of MCF-7 cells using anti-XRCC6 antibody (PA1642). Overlay histogram showing MCF-7 cells stained with PA1642 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-XRCC6 Antibody (PA1642, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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