

## Anti-Ku80/XRCC5 Antibody Picoband™

Catalog Number: PB9464

### About XRCC5

XRCC5 (X-ray Repair, Complementing Defective, In Chinese Hamster, 5), also known as Ku80 or Ku86, is a protein that in humans, is encoded by the XRCC5 gene. The XRCC5 gene encodes the 80-kD subunit of the Ku autoantigen, a heterodimer which contributes to genomic integrity through its ability to bind DNA double-strand breaks and facilitate repair by the nonhomologous end joining (NHEJ) pathway. The XRCC5 gene is mapped to 2q35. Human colon cancer cells heterozygous for Ku86 are haploinsufficient with an increase in polyploid cells, a reduction in cell proliferation, elevated p53 levels, and a slight hypersensitivity to ionizing radiation. Functional inactivation of the second Ku86 allele results in cells with a drastically reduced doubling time. The Ku86 locus is essential in human somatic tissue culture cells by experiments demonstration. A rare microsatellite polymorphism in XRCC5 is associated with cancer in patients of varying radiosensitivity.

### Overview

|                      |                                                                                                                                                                                                       |
|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Product Name         | Anti-Ku80/XRCC5 Antibody Picoband™                                                                                                                                                                    |
| Reactive Species     | Human, Mouse, Rat                                                                                                                                                                                     |
| Description          | Boster Bio Anti-Ku80/XRCC5 Antibody Picoband™ catalog # PB9464. Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.                          |
| Application          | Flow Cytometry, IF, IHC, IHC-F, ICC, WB                                                                                                                                                               |
| Clonality            | Polyclonal                                                                                                                                                                                            |
| Formulation          | Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 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           | P13010                                                                                                                                                                                                |

### Technical Details

|                               |                                                                                                                                                                                                  |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Immunogen                     | A synthetic peptide corresponding to a sequence at the C-terminus of human Ku80, different from the related mouse sequence by three amino acids.                                                 |
| Predicted Reactive Species    | Hamster                                                                                                                                                                                          |
| 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), IHC(F) 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, Mouse, Rat</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, By Heat</p> <p>Immunohistochemistry (Frozen Section), 0.5-1ug/ml, Human</p> <p>Immunocytochemistry/Immunofluorescence, 2ug/ml, Human</p> <p>Flow Cytometry, 1-3ug/1x10<sup>6</sup> cells, Human</p> |

## Anti-Ku80/XRCC5 Antibody Picoband™ (PB9464) Images

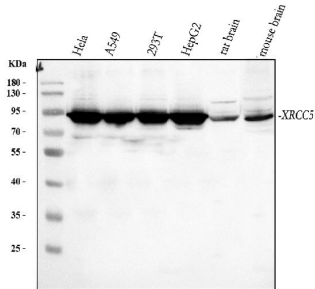


Figure 1. Western blot analysis of KU80 using anti-KU80 antibody (PB9464).

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 HeLa whole cell lysates,  
Lane 2: human A549 whole cell lysates,  
Lane 3: human 293T whole cell lysates,  
Lane 4: human HepG2 whole cell lysates,  
Lane 5: rat brain tissue lysates,  
Lane 6: mouse brain tissue 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-KU80 antigen affinity purified polyclonal antibody (Catalog # PB9464) 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 AKU80 at approximately 83 kDa. The expected band size for KU80 is at 83 kDa.

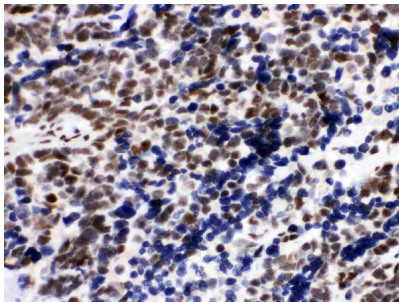


Figure 2. IHC analysis of KU80 using anti-KU80 antibody (PB9464).

KU80 was detected in a paraffin-embedded section of human lung cancer 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-KU80 Antibody (PB9464) 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.

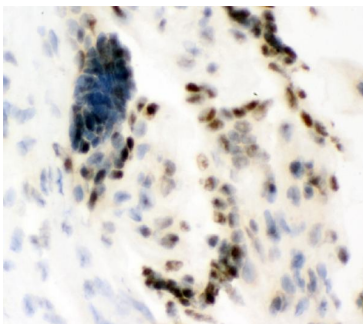
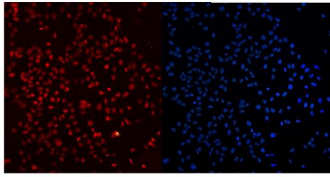


Figure 3. IHC analysis of Ku80 using anti-Ku80 antibody (PB9464).

Ku80 was detected in frozen section of human placenta tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Ku80 Antibody (PB9464) 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 4. IF analysis of Ku80 using anti-Ku80 antibody (PB9464).



Ku80 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 2ug/mL rabbit anti-Ku80 Antibody (PB9464) overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) 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.

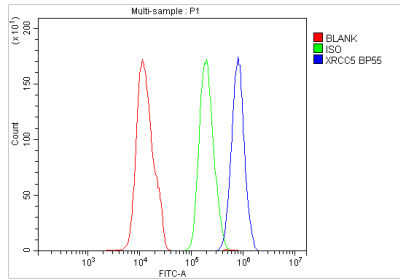


Figure 5. Flow Cytometry analysis of SiHa cells using anti-Ku80 antibody (PB9464).

Overlay histogram showing SiHa cells stained with PB9464 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Ku80 Antibody (PB9464, 1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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Anti-Ku80/XRCC5 Antibody <sup>TM</sup>