

# Affinity-purified Goat Anti-human Ku70/XRCC6 Antibody

## ORDERING INFORMATION

**Catalog Number:** AF5597

**Lot Number:** CCQN01

**Size:** 100 µg

**Storage:** -20° C

**Specificity:** human Ku70

**Immunogen:** *E. coli*-derived rhKu70  
(aa 405 - 556)

**Ig Type:** goat IgG

**Application:** Western blot

## Background

Ku70 (Lupus Ku autoantigen protein 70; also ATP-dependent DNA helicase 2 subunit 1) is a ubiquitous 70 - 75 kDa nuclear protein that serves as a component of the DNA-dependent protein kinase complex DNA-PK. In particular, it heterodimerizes with Ku80 to form a complex that binds DNA ends and select sequences, and which recruits DNA-PKcs for the purpose of phosphorylating multiple chromatin-bound proteins, including p53, Oct-1 and Sp-1. The Ku:DNA-PKcs complex participates in DNA repair, DNA replication, and VDJ recombination. Human Ku70 is 609 amino acids (aa) in length. It contains a Ku core domain (aa 266 - 529), a DNA binding motif (aa 268 - 274), an NLS (aa 539 - 556) and a C-terminal SAP domain (aa 573 - 607). There is one potential splice form that shows a deletion of aa 66 - 106. Over aa 405 - 556, Ku70 shares 83% aa identity with mouse Ku70.

## Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human Ku70 (rhKu70; aa 405 - 556; Accession # P12956). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

## Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

## Reconstitution

Reconstitute in PBS containing 0.02% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.

## Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

## Specificity

This antibody detects endogenous human Ku70 at 70 kDa using Western blot.

## Application

**Western blot** - An antibody concentration of 1.0 µg/mL is recommended.

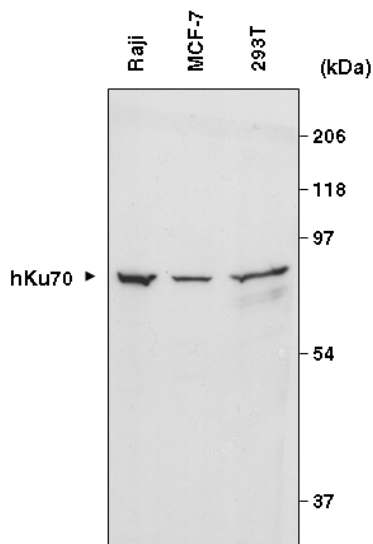
### Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween <sup>®</sup> 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane 1 hour at room temperature in Antibody Solution containing 1.0 µg/mL goat anti-human Ku70.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:2,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

**Cell lysates for Western blottings** - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at  $2 \times 10^6$  -  $1 \times 10^7$  cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each.

**Optimal dilutions should be determined by each laboratory for each application.**



### Detection of hKu70 with AF5597.

Lysates from human Raji, MCF7, and HEK293T cells were resolved by SDS-PAGE. Following electrophoresis, lysates were transferred to a PVDF membrane and immunoblotted with 1.0 µg/mL anti-hKu70, as described in *Protocols for Immunoblotting*.