

Product datasheet

Anti-Ku70 antibody [EPR4027] ab92450

Recombinant RabMAb

★★★★★ 1 Abreviews 14 References 10 Images

Overview

Properties

Product name	Anti-Ku70 antibody [EPR4027]		
Description	Rabbit monoclonal [EPR4027] to Ku70		
Host species	Rabbit		
Tested applications	Suitable for: WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)		
Species reactivity	Reacts with: Human		
Immunogen	Synthetic peptide within Human Ku70 aa 500-600. The exact sequence is proprietary. Database link: P12956		
Positive control	WB: A549, 293T, A431, and HeLa lysates. IHC-P: human colon carcinoma, tonsil and testis tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: HeLa cells.		
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including:		
	- High batch-to-batch consistency and reproducibility		
	- Improved sensitivity and specificity		
	- Long-term security of supply		
	- Animal-free production		
	For more information <u>see here</u> .		
	Our RabMAb $^{ extsf{B}}$ technology is a patented hybridoma-based technology for making rabbit		
	monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u> .		
	Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.		

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR4027
lsotype	lgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab92450 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★ ★ <u>(1)</u>	1/1000 - 1/10000. Predicted molecular weight: 70 kDa.
IP		1/20.
IHC-P		 1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Antigen retrieval and the use of an HRP/AP polymerized secondary antibody is recommended for enhanced staining. See <u>IHC antigen retrieval protocols</u>.
ICC/IF		1/100 - 1/250.
Flow Cyt (Intra)		1/10 - 1/100. <u>ab172730</u> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

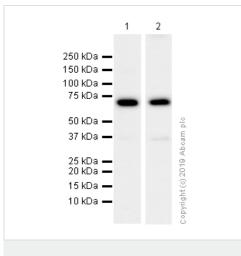
Function	Single stranded DNA-dependent ATP-dependent helicase. Has a role in chromosome translocation. The DNA helicase II complex binds preferentially to fork-like ends of double-stranded DNA in a cell cycle-dependent manner. It works in the 3'-5' direction. Binding to DNA may be mediated by XRCC6. Involved in DNA non-homologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination. The XRCC5/6 dimer acts as regulatory subunit of the DNA-dependent protein kinase complex DNA-PK by increasing the affinity of the catalytic subunit PRKDC to DNA by 100-fold. The XRCC5/6 dimer is probably involved in stabilizing broken DNA ends and bringing them together. The assembly of the DNA-PK complex to DNA ends is required for the NHEJ ligation step. Required for osteocalcin gene expression. Probably also acts as a 5'-deoxyribose-5-phosphate lyase (5'-dRP lyase), by catalyzing the beta-elimination of the 5' deoxyribose-5-phosphate at an abasic site near double-strand breaks. 5'-dRP lyase activity allows to 'clean' the termini of abasic sites, a class of nucleotide damage commonly associated with strand breaks, before such broken ends can be joined. The XRCC5/6 dimer together with APEX1 acts as a negative regulator of transcription.
Sequence similarities	Belongs to the ku70 family. Contains 1 Ku domain. Contains 1 SAP domain.
Developmental stage	Expression does not increase during promyelocyte differentiation.
Post-translational	Phosphorylation by PRKDC may enhance helicase activity. Phosphorylation of Ser-51 does not

modifications

Cellular localization

affect DNA repair. Nucleus. Chromosome.

Images



Western blot - Anti-Ku70 antibody [EPR4027] (ab92450)

All lanes : Anti-Ku70 antibody [EPR4027] (ab92450) at 1/5000 dilution (Purified)

Lane 1 : A549 (Human lung carcinoma epithelial cell) whole cell lysates

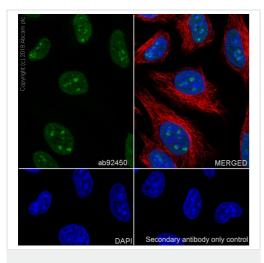
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

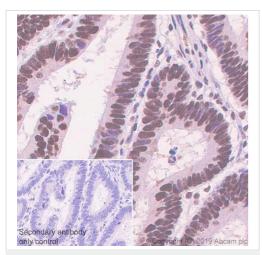
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 70 kDa Observed band size: 70 kDa

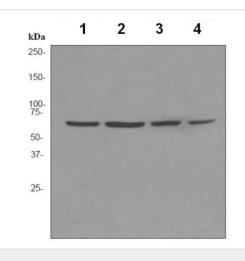


Immunocytochemistry/ Immunofluorescence - Anti-Ku70 antibody [EPR4027] (ab92450)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Ku70 with purified ab92450 at 1/250 dilution (0.46 µg/ml). Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] -Microtubule Marker (Alexa Fluor[®] 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor[®] 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ku70 antibody [EPR4027] (ab92450)



Western blot - Anti-Ku70 antibody [EPR4027] (ab92450) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon carcinoma tissue sections labeling Ku70 with purified ab92450 at 1/250 dilution (0.46 µg/ml). Heat mediated antigen retrieval using Bond[™] Epitope Retrieval Solution 1 (pH 6.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody.

Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

All lanes : Anti-Ku70 antibody [EPR4027] (ab92450) at 1/1000 dilution ((unpurified))

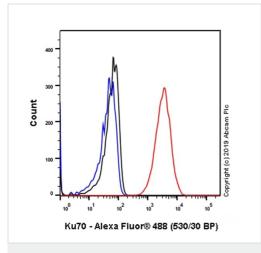
Lane 1 : HeLa cell lysate Lane 2 : 293T cell lysate Lane 3 : A549 cell lysate Lane 4 : A431 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

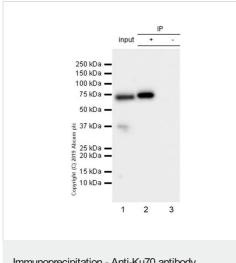
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 70 kDa



Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Ku70 with purified ab92450 at 1/20 dilution (5µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

Flow Cytometry (Intracellular) - Anti-Ku70 antibody [EPR4027] (ab92450)



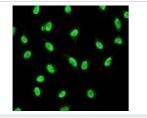
Immunoprecipitation - Anti-Ku70 antibody [EPR4027] (ab92450) ab92450 (purified) at 1/20 dilution (0.5ug) immunoprecipitating Ku70 in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug Lane 2 (+): ab92450 & HeLa whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab92450 in HeLa whole cell lysate

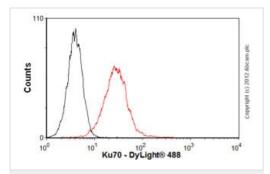
For western blotting, VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) was used at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.

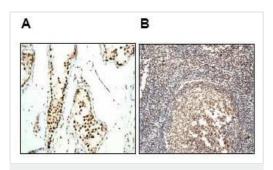


Immunofluorescence staining of HeLa cells using unpurified ab92450 at 1/100 dilution.

Immunocytochemistry/ Immunofluorescence - Anti-Ku70 antibody [EPR4027] (ab92450)

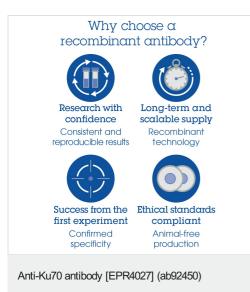


Flow Cytometry (Intracellular) - Anti-Ku70 antibody [EPR4027] (ab92450) Overlay histogram showing HeLa cells stained with unpurified ab92450 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab92450, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight[®] 488 goat antirabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ku70 antibody [EPR4027] (ab92450) Paraffin embedded Human testis tissue (A) or Human tonsil tissue (B) were labelled with unpurified ab92450 at 1/100 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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