abcam

ab80592

Anti-Ku80 antibody [EPR3468]

Anti-Ku80 antibody [EPR3468] (ab80592) is a rabbit monoclonal antibody detecting Ku80 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 40 publications
- Trusted since 2009

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR3468
Purification technique	Affinity purification Protein A
Concentration	0.102 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Rat
Dilution info	1/250 - 1/500
Notes	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Mouse
Dilution info	1/250 - 1/500
Notes	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/250 - 1/500
Notes	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat. Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

IP

Tested

Species	Human
Dilution info	1/50
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/10000
Notes	-

Species	Rat
Dilution info	1/1000 - 1/10000
Notes	-

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500 - 1/1000
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information XRCC5

Function	DNA-binding protein critical for the DNA damage response, specifically in repairing double-strand breaks (DSBs) via the classical non-homologous end joining (NHEJ) pathway. It forms a heterodimer with XRCC6 (Ku70), creating the Ku70:Ku80 heterodimer (Ku complex), which serves as a DNA end-binding complex. It primarily binds DSBs and recruits essential repair factors, assembling the core long-range NHEJ complex to facilitate the alignment and ligation of broken DNA ends (PubMed:11493912, PubMed:33854234, PubMed:34352203). This pathway ensures the rapid repair of cytotoxic and mutagenic DSBs and contributes to the generation of diversity in T-cell receptors and antibodies through mechanisms such as V(D)J recombination (PubMed:9742108). Likely acts as a 5'-deoxyribose-5-phosphate lyase (5'-dRP lyase), catalyzing the beta-elimination of the 5'-deoxyribose-5-phosphate at abasic sites near DSBs. This activity cleans the termini of abasic sites, a common form of nucleotide damage, preparing broken ends for ligation (PubMed:20383123). It may also possess 3'-5' DNA helicase activity, although this has not been confirmed in vivo, and its physiological significance remains unclear (PubMed:7957065). Beyond DNA repair, the protein contributes to telomere maintenance (PubMed:29490055). It is also implicated in transcriptional regulation, acting as a cofactor for various transcription factors (PubMed:12145306, PubMed:8621488). It plays a role in the regulation of DNA virus-mediated innate immune response by assembling into the HDP-RNP complex, a complex that serves as a platform for IRF3 phosphorylation and subsequent innate immune response activation through the cGAS-STING pathway (PubMed:28712728). Can also bind RNAs and recruits PRKDC to a wide range of cellular RNAs, including the U3 small nucleolar RNA, playing a role in the biogenesis of ribosomal RNAs (PubMed:32103174).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

Aliquoting information	Upon delivery aliquot
Storage information	Stable for 12 months at -20°C

Notes

What is this antibody validated in?
Anti-Ku80 antibody [EPR3468] (ab80592) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Mouse, Rat samples.

What is the molecular weight of Ku80?
Anti-Ku80 [EPR3468] (ab80592) specifically detects a band for Ku80 (UniProt: P13010) at a molecular weight of 83kDa.

Trusted by the scientific community
Anti-Ku80 [EPR3468] (ab80592) was first used in a scientific publication in 2009 and has been cited over 40 times in peer-reviewed journals.

Other related products
We have a range of other formats of antibody clone [EPR3468] also available for your convenience: ab80592, Alexa Fluor® 488 - ab198586, Alexa Fluor® 647 - ab198587, HRP - ab199096, PE - ab225046, Carrier free - ab232381

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The Ku80 protein also known as Ku p70 Ku antigen p70 and by gene identifiers such as Ku80 hum39 and 5c5 functions critically in DNA repair processes. With a molecular mass of approximately 80 kDa Ku80 is ubiquitously expressed in mammalian cells. It is an important component of the Ku heterodimer working with its partner Ku70 to detect and bind DNA double-strand breaks (DSBs). This

	action initiates repair by aligning with DNA ends to prevent break processing and sustain genome integrity.
Biological function summary	The Ku80 protein executes an important role in maintaining cellular stability by participating in the non-homologous end joining (NHEJ) pathway. Ku80 forms a complex with DNA-PKcs (DNA-dependent protein kinase catalytic subunit) which activates the kinase to phosphorylate itself and other proteins facilitating the repair of DSBs. Its expression is significant in tissues that undergo high rates of cellular turnover and are experienced with DNA damage where the DNA repair mechanisms are important for regular cell cycle progression.
Pathways	DNA repair and V(D)J recombination are principal domains where Ku80 shows its influence. In the context of DNA repair Ku80's partnership with DNA-PKcs as part of the NHEJ repair pathway is fundamental in fixing DNA damage from environmental insults or normal cellular metabolism. In the immune development process through V(D)J recombination Ku80 partners with other proteins like the Artemis complex to perform programmed DNA rearrangements required for the diversity of immune receptors.
Associated diseases and disorders	Ku80 has a significant relation to cancer and immune deficiencies. Defects in Ku80 function can lead to increased sensitivity to ionizing radiation and a higher propensity for cancer development because of impaired DNA repair capabilities. Moreover in immune-related disorders inadequate Ku80 performance affects the maturation of lymphocytes leading to immune system malfunction. Its malfunctioning connection with proteins like DNA-PKcs and Artemis highlights its critical roles in both tumor suppression and immune system competence.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

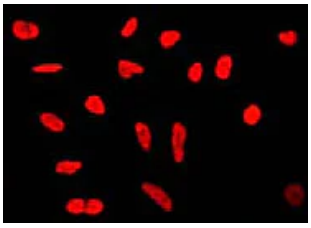
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

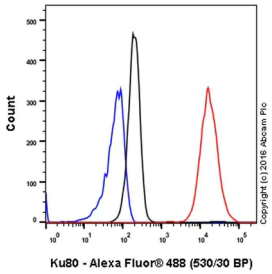
8 product images



Immunocytochemistry/ Immunofluorescence - Anti-Ku80 antibody [EPR3468] (ab80592)

Ku80 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ku80 antibody

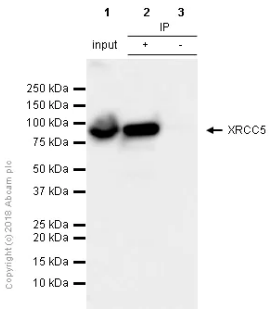
Immunofluorescence analysis of HeLa cells with 1/500 ab80592.



Flow Cytometry (Intracellular) - Anti-Ku80 antibody [EPR3468] (ab80592)

Ku80 Flow Cytometry (Intracellular) staining using rabbit Anti-Ku80 antibody

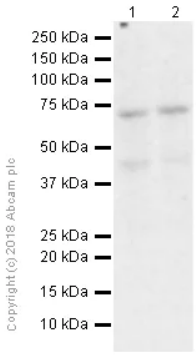
Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Ku80 with unpurified ab80592 at 1/20 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Immunoprecipitation - Anti-Ku80 antibody [EPR3468] (ab80592)

ab80592 (purified) at 1/500 dilution (1.86 ©:g/ml) immunoprecipitating Ku80 in HeLa whole cell lysate.
Lane 1 (input): HeLa(Human cervix adenocarcinoma epithelial cell) whole cell lysate 10©:g
Lane 2 (+): ab80592 & HeLa whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab80592 in HeLa whole cell lysate
For western blotting, VeriBlot for IP secondary antibody (HRP) (ab131366) was used as the secondary antibody at 1/1000 dilution.
Blocking and diluting buffer: 5% NFDM /TBST .
All lanes:
Immunoprecipitation - Anti-Ku80 antibody [EPR3468] (ab80592)

Predicted band size: 83 kDa



Western blot - Anti-Ku80 antibody [EPR3468] (ab80592)

All lanes:
Western blot - Anti-Ku80 antibody [EPR3468] (ab80592) at 1/3000 dilution

Lane 1:
PC-12(Rat adrenal gland pheochromocytoma) whole cell lysate at 20 µg

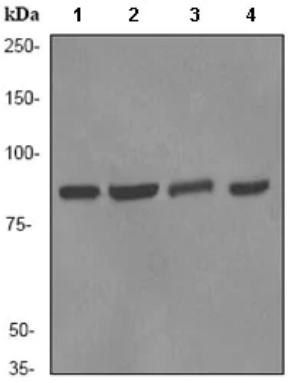
Lane 2:
NIH/3T3(Mouse embryonic fibroblast) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 83 kDa

Observed band size: 83 kDa



Western blot - Anti-Ku80 antibody [EPR3468] (ab80592)

All lanes:
Western blot - Anti-Ku80 antibody [EPR3468] (ab80592) at 1/50000 dilution

Lane 1:
A549 cell lysate at 10 µg

Lane 2:
HeLa cell lysate at 10 µg

Lane 3:
HepG2 cell lysate at 10 µg

Lane 4:
MCF7 cell lysate at 10 µg

Secondary

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

Predicted band size: 83 kDa

Observed band size: 83 kDa

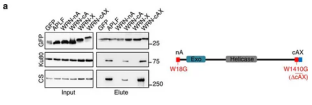


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Western blot - Anti-Ku80 antibody [EPR3468] (ab80592)

Ku80 Western blot staining using rabbit Anti-Ku80 antibody

Western Blotting using Anti-Ku80 antibody [EPR3468], ab80592. Publication image from Rulten, S. L. et al., 2016, Nat Commun, 27063109. Legend direct from paper.

The WRN C-terminal KBM and XLF-like motif bind Ku protein complexes cooperatively.(a) HEK293T cells were co-transfected with expression constructs encoding GFP or the indicated GFP-tagged KBMs and GFP-tagged proteins recovered using GFP-TRAP beads. Aliquots of the input and eluate samples were fractionated by SDS–PAGE and immunoblotted for GFP, Ku80 and DNA–PKcs (CS). Right, cartoon depicting WRN and the position of the KBMs and XLF-like motif and the mutations employed in these experiments. (b) HEK293T cells were transfected with expression constructs encoding the indicated wild-type or mutated GFP-tagged WRN C-terminal KBM, XLF-like motif (‘X'), or KBM plus XLF-like motif in tandem. Cells were micro-irradiated with UVA as in Fig. 2. Representative images (left) and quantification (right) are shown. All quantified data are the mean GFP fluorescence (±s.e.m.) in the laser track relative to the mean GFP fluorescence before irradiation (set at 100%) from >20 cells per experiment. (c,d) Expression constructs encoding full-length wild-type (‘WT') GFP–WRN or derivatives harbouring the indicated point mutations in the N-terminal KBM (W18G), C-terminal KBM (W1410G) or deleted C-terminal tandem domain (δcAX) or XLF-like motif (δX) were transfected into HEK293T cells and recovered using GFP-TRAP beads. Input and eluates were immunoblotted for GFP and Ku80. Numbers in parentheses are the fraction of Ku co-precipitated by the indicated GFP-tagged WRN protein, relative to wild-type WRN,

quantified by ImageJ. Data are from two to six independent experiments, except for W18G/δcAX in which Ku recovery was too low to be determined (‘nd'). (e) Direct interaction of purified full-length Strep-tagged WRN with recombinant human Ku. Recombinant Strep-tagged WRN, WRNδcAX or WRNW18G was immobilized on Streptavidin Mag sepharose beads and incubated with recombinant Ku heterodimer. Aliquots of the recombinant proteins employed in the experiment are shown on the left (lanes 1–3) and proteins pulled down by the indicated Strep-tagged WRN protein are shown on the right (lanes 4–7). Lane 6 contains the proteins recovered in a control pull-down that lacked Strep-tagged WRN. Proteins were fractioned by SDS–PAGE and stained with Coomassie Blue.

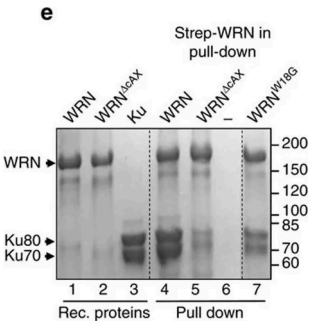


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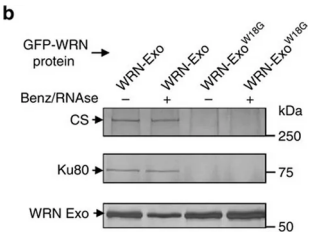


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The WRN N-terminal KBM promotes WRN exonuclease activity.(a) Left, cartoon illustrating the GFP-tagged truncated recombinant WRN proteins employed in these experiments. The WRN N-terminal (‘nA') and C-terminal (‘cA') KBMs are indicated by red boxes and XLF-like motif (‘X') by a blue box. The exonuclease domain is indicated by a black box, and the position of the KBM mutation (W18G) by an asterisk and dotted line. Middle, U2-OS cells transiently expressing the indicated recombinant GFP-tagged WRN protein were imaged for GFP before and after UVA microirradiation,

as in Fig. 2. Right, Ku80−/− MEFs transiently co-expressing GFP-tagged WRN-Exo, RFP-Ku70, and either RFP (vector), RFP-Ku80 or RFP-Ku80L68R as indicated were micro-irradiated as in Fig. 1. Data are the mean GFP fluorescence (±s.e.m.) in the laser track relative to the mean GFP fluorescence before irradiation (set at 100%) from >20 cells per experiment. (b) The indicated GFP-tagged WRN proteins were recovered from transiently transfected HEK293T cell lysates pre-treated or not as indicated with Benzonase and RNAse in pull-down assays using GFP-TRAP beads. Aliquots of the bead eluate were fractionated by SDS-PAGE and silver stained to detect GFP-WRN, GFP-WRNW18G, Ku80, and DNA-PKcs (‘CS'). (c) Cy3-labeled 30 bp duplex oligonucleotide (20 nM) with a 5′ overhang was incubated with 500, 100, 20 or 5 nM HIs-tagged WRN-Exo or WRN-ExoW18G in the absence or presence of 100 nM Ku heterodimer (Ku70/Ku80, ‘Ku') and 5 mM MgCl2. Exonuclease products were resolved on a 16% TBE-Urea gel. (d) Exonuclease assays were conducted as above in the presence of 5 mM MgCl2 using 10 nM His-tagged WRN-Exo and 100, 20, 4 or 0.8 nM of either Ku heterodimer (Ku70/Ku80; ‘Ku'), KuδC heterodimer (Ku70/Ku80δC; ‘KuδC'), or mutant KuδC heterodimer harbouring the Ku80 mutation, L68R (KuδCL68R). (e) Exonuclease assays were conducted as above using 100, 20 and 4 nM of the indicated His-tagged WRN protein and 10 nM wild-type Ku heterodimer (Ku70/Ku80; ‘Ku') in 5 mM Mg2+.

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