abcam

Product datasheet

Anti-HMGB2 antibody [EPR6301] ab124670

KO VALIDATED Recombinant RabMAb

★★★★★ 2 Abreviews 14 References 7 Images

Overview

Product name	Anti-HMGB2 antibody [EPR6301]
Description	Rabbit monoclonal [EPR6301] to HMGB2
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, WB, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide within Human HMGB2 (N terminal). The exact sequence is proprietary.
Positive control	WB: HEK-293T, HAP1, K562, HeLa and PC12 cell lysates. ICC/IF: PC-12 cells. IHC-P: Human breast tissue.
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[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>.

Properties

Form	Liquid		
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.		
Dissociation constant (K _D)	$K_{D} = 6.03 \times 10^{-10} M$		
	LOW 10 ⁻⁶ -7 -8 -9 -10 -11 -12 Learn more about K _D		

Storage buffer

pH: 7.20

Preservative: 0.05% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture

	supernatant
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR6301
Isotype	lgG

Applications

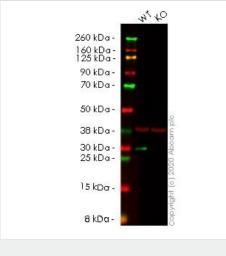
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab124670 in the following tested applications.

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

Application	Abreviews	Notes
ICC/IF		1/500.
WB	★★★★★(1)	1/10000 - 1/50000. Predicted molecular weight: 24 kDa.
IHC-P		1/250 - 1/500. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

Target	
Function	DNA binding proteins that associates with chromatin and has the ability to bend DNA. Binds preferentially single-stranded DNA. Involved in V(D)J recombination by acting as a cofactor of the RAG complex. Acts by stimulating cleavage and RAG protein binding at the 23 bp spacer of conserved recombination signal sequences (RSS).
Sequence similarities	Belongs to the HMGB family. Contains 2 HMG box DNA-binding domains.
Cellular localization	Nucleus. Chromosome.

Images



Western blot - Anti-HMGB2 antibody [EPR6301] (ab124670) All lanes : Anti-HMGB2 antibody [EPR6301] (ab124670) at 1/2000 dilution

Lane 1 : Wild-type HEK-293T cell lysate Lane 2 : HMGB2 knockout HEK-293T cell lysate

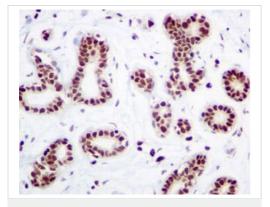
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 24 kDa Observed band size: 24 kDa

Lanes 1-2: Merged signal (red and green). Green - ab124670 observed at 24 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) observed at 37 kDa.

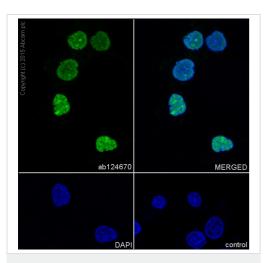
ab124670 was shown to react with HMGB2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266358** (knockout cell lysate **ab257156**) was used. Wild-type HEK-293T and HMGB2 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab124670 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®]680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



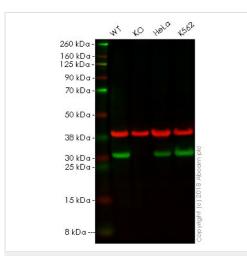
ab124670, at a 1/250 dilution, staining HMGB2 in paraffin embedded Human breast tissue by Immunohistochemistry.

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HMGB2 antibody [EPR6301] (ab124670)



Immunocytochemistry/ Immunofluorescence - Anti-HMGB2 antibody [EPR6301] (ab124670) Immunocytochemistry/Immunofluorescence analysis of PC-12 cells labelling HMGB2 with ab124670 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Control: PBS only. Nuclear counter stain: DAPI.



Western blot - Anti-HMGB2 antibody [EPR6301] (ab124670) All lanes : Anti-HMGB2 antibody [EPR6301] (ab124670) at 1/10000 dilution

- Lane 1 : Wild-type HAP1 whole cell lysate
- Lane 2 : HMGB2 knockout HAP1 whole cell lysate
- Lane 3 : HeLa whole cell lysate
- Lane 4 : K562 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 24 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab124670 observed at 24 kDa. Red - loading control, <u>ab9484</u>, observed at 37 kDa.

ab124670 was shown to specifically react with HMGB2 in wild-type HAP1 cells as signal was lost in HMGB2 knockout cells. Wild-type and HMGB2 knockout samples were subjected to SDS-PAGE. Ab124670 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes : Anti-HMGB2 antibody [EPR6301] (ab124670) at 1/10000 dilution

Lane 1 : K562 cell lysates Lane 2 : HeLa cell lysates Lane 3 : PC12 cell lysates

Lysates/proteins at 10 µg per lane.

Secondary

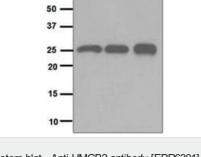
All lanes : Goat-anti-rabbit HRP at 1/2000 dilution

Predicted band size: 24 kDa

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about KD



1

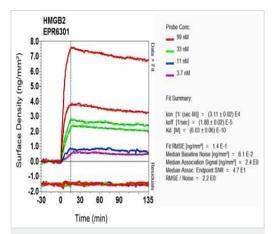
250

150

2

3

Western blot - Anti-HMGB2 antibody [EPR6301] (ab124670)



OI-RD Scanning - Anti-HMGB2 antibody [EPR6301] (ab124670)



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