# abcam

# Product datasheet

# Anti-HMGB2 antibody [EPR6302] - BSA and Azide free ab248543



Recombinant

RabMAb

# 3 Images

#### Overview

Product name Anti-HMGB2 antibody [EPR6302] - BSA and Azide free

**Description** Rabbit monoclonal [EPR6302] to HMGB2 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IHC-P, WB

Unsuitable for: ICC/IF or IP

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

**Positive control** WB: HEK-293T, HAP1, HeLa and K562 cell lysate.

**General notes** ab248543 is the carrier-free version of <u>ab133540</u>.

Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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 see here.

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

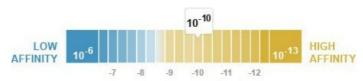
1

# **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

**Dissociation constant (K<sub>D</sub>)**  $K_D = 2.97 \times 10^{-10} M$ 



Learn more about K<sub>D</sub>

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR6302

**Isotype** IgG

## **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab248543 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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Predicted molecular weight: 24 kDa.

**Application notes** Is unsuitable for ICC/IF or IP.

**Target** 

**Function** DNA binding proteins that associates with chromatin and has the ability to bend DNA. Binds

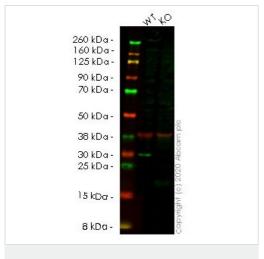
preferentially single-stranded DNA. Involved in V(D)J recombination by acting as a cofactor of the RAG complex. Acts by stimulating cleavage and RAG protein binding at the 23 bp spacer of

conserved recombination signal sequences (RSS).

**Sequence similarities** Belongs to the HMGB family.

Contains 2 HMG box DNA-binding domains.

**Cellular localization** Nucleus. Chromosome.



Western blot - Anti-HMGB2 antibody [EPR6302] - BSA and Azide free (ab248543)

**All lanes :** Anti-HMGB2 antibody [EPR6302] (<u>ab133540</u>) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: HMGB2 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

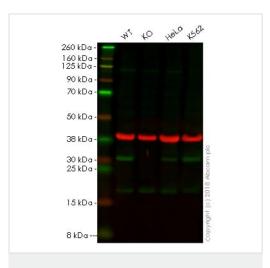
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (<u>ab133540</u>).

Lanes 1-2: Merged signal (red and green). Green - <u>ab133540</u> observed at 24 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) observed at 37 kDa.

ab133540 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. ab133540 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-HMGB2 antibody [EPR6302] - BSA and Azide free (ab248543)

**All lanes :** Anti-HMGB2 antibody [EPR6302] (<u>ab133540</u>) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: HMGB2 knockout HAP1 whole cell lysate

Lane 3 : HeLa cell lysate Lane 4 : K562 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 24 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab133540</u>).

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab133540</u> observed at 24 kDa. Red - loading control, <u>ab9484</u>, observed at 37 kDa.

ab133540 was shown to recognize HMGB2 in wild-type HAP1 cells as signal was lost at the expected MW in HMGB2 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and HMGB2 knockout samples were subjected to SDS-PAGE. Ab133540 and ab9484 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1000 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.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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