abcam

Product datasheet

Anti-APE1 antibody [13B8E5C2] ab194

KO VALIDATED

10 Abreviews 44 References 3 Images

Overview

Product name	Anti-APE1 antibody [13B8E5C2]
Description	Mouse monoclonal [13B8E5C2] to APE1
Host species	Mouse
Specificity	This antibody is specific to the human APE/ref-1 protein.
Tested applications	**Suitable for:** WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Full length native protein (purified) (Human).
Positive control	WB: HepG2, HEK293 and wild-type HAP1 cell lysates. IHC-P: Human normal placenta tissue.
General notes	APE appears to form a unique link between the DNA base excision pathway, oxidative signalling, trancription regulation, cancer and cell-cycle control. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituent: PBS
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	13B8E5C2
Myeloma	unknown

Isotype	IgG2b
Light chain type	unknown

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab194 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	★★★★★ **(4)**	1/2000. Predicted molecular weight: 35.6 kDa.
IHC-P		1/5000.

Target

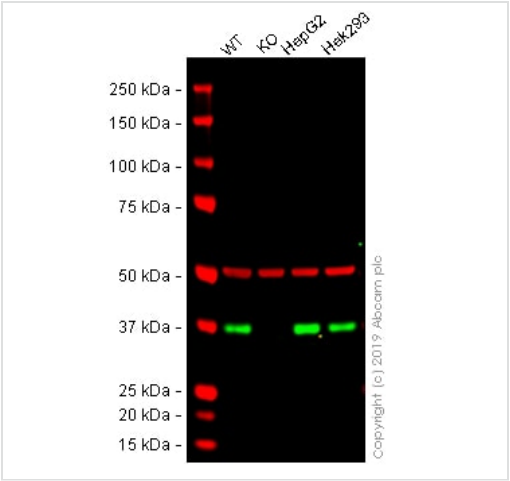
Function Multifunctional protein that plays a central role in the cellular response to oxidative stress. The two major activities of APEX1 in DNA repair and redox regulation of transcriptional factors. Functions as a apurinic/apyrimidinic (AP) endodeoxyribonuclease in the DNA base excision repair (BER) pathway of DNA lesions induced by oxidative and alkylating agents. Initiates repair of AP sites in DNA by catalyzing hydrolytic incision of the phosphodiester backbone immediately adjacent to the damage, generating a single-strand break with 5'-deoxyribose phosphate and 3'-hydroxyl ends. Does also incise at AP sites in the DNA strand of DNA/RNA hybrids, single-stranded DNA regions of R-loop structures, and single-stranded RNA molecules. Has a 3'-5' exoribonuclease activity on mismatched deoxyribonucleotides at the 3' termini of nicked or gapped DNA molecules during short-patch BER. Possesses a DNA 3' phosphodiesterase activity capable of removing lesions (such as phosphoglycolate) blocking the 3' side of DNA strand breaks. May also play a role in the epigenetic regulation of gene expression by participating in DNA demethylation. Acts as a loading factor for POLB onto non-incised AP sites in DNA and stimulates the 5'-terminal deoxyribose 5'-phosphate (dRp) excision activity of POLB. Plays a role in the protection from granzymes-mediated cellular repair leading to cell death. Also involved in the DNA cleavage step of class switch recombination (CSR). On the other hand, APEX1 also exerts reversible nuclear redox activity to regulate DNA binding affinity and transcriptional activity of transcriptional factors by controlling the redox status of their DNA-binding domain, such as the FOS/JUN AP-1 complex after exposure to IR. Involved in calcium-dependent down-regulation of parathyroid hormone (PTH) expression by binding to negative calcium response elements (nCaREs). Together with HNRNPL or the dimer XRCC5/XRCC6, associates with nCaRE, acting as an activator of transcriptional repression. Stimulates the YBX1-mediated MDR1 promoter activity, when acetylated at Lys-6 and Lys-7, leading to drug resistance. Acts also as an endoribonuclease involved in the control of single-stranded RNA metabolism. Plays a role in regulating MYC mRNA turnover by preferentially cleaving in between UA and CA dinucleotides of the MYC coding region determinant (CRD). In association with NMD1, plays a role in the rRNA quality control process during cell cycle progression. Associates, together with YBX1, on the MDR1 promoter. Together with NPM1, associates with rRNA. Binds DNA and RNA.

Sequence similarities Belongs to the DNA repair enzymes AP/ExoA family.

Domain The N-terminus contains the redox activity while the C-terminus exerts the DNA AP-endodeoxyribonuclease activity; both function are independent in their actions. An unconventional mitochondrial targeting sequence (MTS) is harbored within the C-terminus, that appears to be

	masked by the N-terminal sequence containing the nuclear localization signal (NLS), that probably blocks the interaction between the MTS and Tom proteins.
Post-translational modifications	Phosphorylated. Phosphorylation by kinase PKC or casein kinase CK2 results in enhanced redox activity that stimulates binding of the FOS/JUN AP-1 complex to its cognate binding site. AP-endodeoxyribonuclease activity is not affected by CK2-mediated phosphorylation. Phosphorylation of Thr-233 by CDK5 reduces AP-endodeoxyribonuclease activity resulting in accumulation of DNA damage and contributing to neuronal death. Acetylated on Lys-6 and Lys-7. Acetylation is increased by the transcriptional coactivator EP300 acetyltransferase, genotoxic agents like H(2)O(2) and methyl methanesulfonate (MMS). Acetylation increases its binding affinity to the negative calcium response element (nCaRE) DNA promoter. The acetylated form induces a stronger binding of YBX1 to the Y-box sequence in the MDR1 promoter than the unacetylated form. Deacetylated on lysines. Lys-6 and Lys-7 are deacetylated by SIRT1. Cleaved at Lys-31 by granzyme A to create the mitochondrial form; leading in reduction of binding to DNA, AP endodeoxynuclease activity, redox activation of transcription factors and to enhanced cell death. Cleaved by granzyme K; leading to intracellular ROS accumulation and enhanced cell death after oxidative stress. Cys-65 and Cys-93 are nitrosylated in response to nitric oxide (NO) and lead to the exposure of the nuclear export signal (NES). Ubiquitinated by MDM2; leading to translocation to the cytoplasm and proteasomal degradation.
Cellular localization	Mitochondrion. The cleaved APEX2 is only detected in mitochondria (By similarity). Translocation from the cytoplasm to the mitochondria is mediated by ROS signaling and cleavage mediated by granzyme A. Tom20-dependent translocated mitochondrial APEX1 level is significantly increased after genotoxic stress and Nucleus. Nucleus, nucleolus. Nucleus speckle. Endoplasmic reticulum. Cytoplasm. Detected in the cytoplasm of B-cells stimulated to switch (By similarity). Colocalized with SIRT1 in the nucleus. Colocalized with YBX1 in nuclear speckles after genotoxic stress. Together with OGG1 is recruited to nuclear speckles in UVA-irradiated cells. Colocalized with nucleolin and NPM1 in the nucleolus. Its nucleolar localization is cell cycle dependent and requires active rRNA transcription. Colocalized with calreticulin in the endoplasmic reticulum. Translocation from the nucleus to the cytoplasm is stimulated in presence of nitric oxide (NO) and function in a CRM1-dependent manner, possibly as a consequence of demasking a nuclear export signal (amino acid position 64-80). S-nitrosylation at Cys-93 and Cys-310 regulates its nuclear-cytosolic shuttling. Ubiquitinated form is localized predominantly in the cytoplasm.

Images



Western blot - Anti-APE1 antibody [13B8E5C2] - ChIP Grade (ab194)

All lanes : Anti-APE1 antibody [13B8E5C2] (ab194) at 1/2000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : APEX1 knockout HAP1 whole cell lysate
Lane 3 : HepG2 whole cell lysate
Lane 4 : HEK293 whole cell lysate

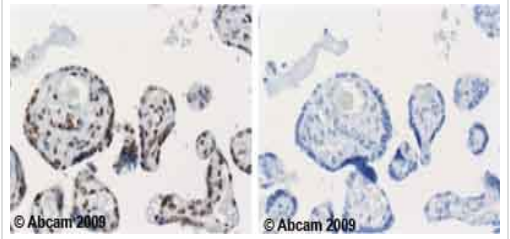
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 35.6 kDa
Observed band size: 37 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab194 observed at 37 kDa. Red - loading control, **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

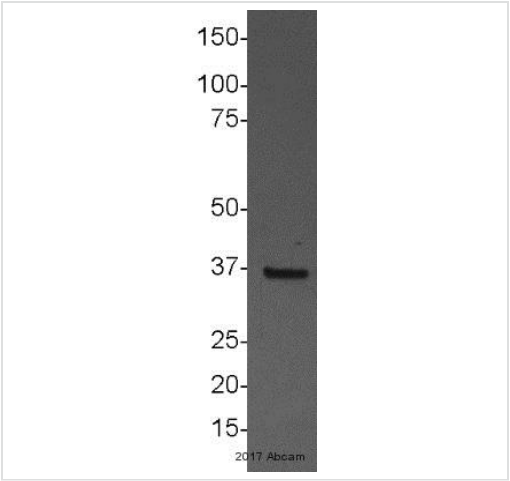
ab194 was shown to react with APEX1 in HAP1 wild-type cells in Western blot. Loss of signal was observed when APEX1 knockout sample was used. HAP1 wild-type and APEX1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween®) before incubation with ab194 and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [13B8E5C2] - ChIP Grade (ab194)

Ab194 staining Human normal placenta. Staining is localized to the nucleus.
Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus , at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 AR buffer citrate pH 6.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS), then incubated with primary antibody for 20 minutes, and detected with Dako Envision Flex amplification kit for 30 minutes.

Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



Western blot - Anti-APE1 antibody [13B8E5C2] - ChIP Grade (ab194)

This image is courtesy of an anonymous Abreview

Anti-APE1 antibody [13B8E5C2] (ab194) at 1/2000 dilution + Human fibroblast at 30 µg with Milk at 5 %

Secondary

ECL Mouse IgG,HRP - linked whole antibody (sheep) at 1/5000 dilution

Developed using the ECL technique.

Predicted band size: 35.6 kDa

Exposure time: 10 seconds

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors