abcam

Product datasheet

Anti-APE1 antibody [EPR18378-45] - BSA and Azide free ab227062

Recombinant RabMAb

7 Images

Overview

Product name	Anti-APE1 antibody [EPR18378-45] - BSA and Azide free
Description	Rabbit monoclonal [EPR18378-45] to APE1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, Flow Cyt, ICC/IF, ChIP, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment within Mouse APE1 aa 50 to the C-terminus. The exact sequence is proprietary. Database link: P28352
Positive control	IHC-P: Human ovarian carcinoma tissue.
General notes	Ab227062 is the carrier-free version of ab189474. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits 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. ab227062 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.

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 long

	term. Avoid freeze / thaw cycle.
Storage buffer	Constituent: PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18378-45
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab227062** 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		Use at an assay dependent concentration. Predicted molecular weight: 35 kDa.
Flow Cyt		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

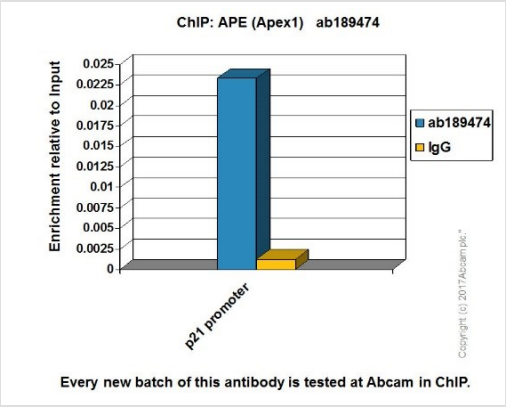
Target

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

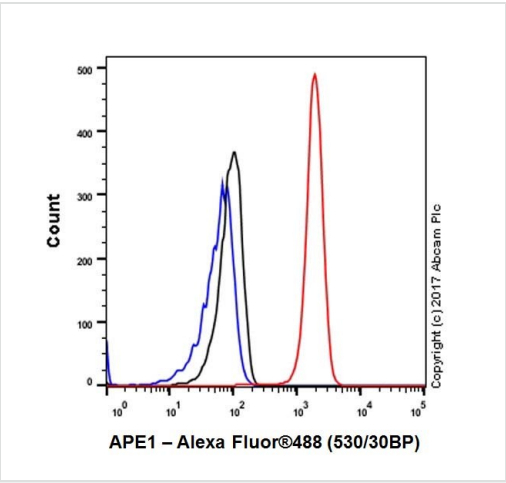


ChIP - Anti-APE1 antibody [EPR18378-45] - BSA and Azide free (ab227062)

Chromatin was prepared from HCT 116 cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab189474 (blue), and 20µl of Protein A/G sepharose beads slurry (10µl of sepharose A beads + 10µl of sepharose G beads). 5µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (SYBR green approach).

ChIP was performed according to the literature (PMID: 23874636).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab189474).

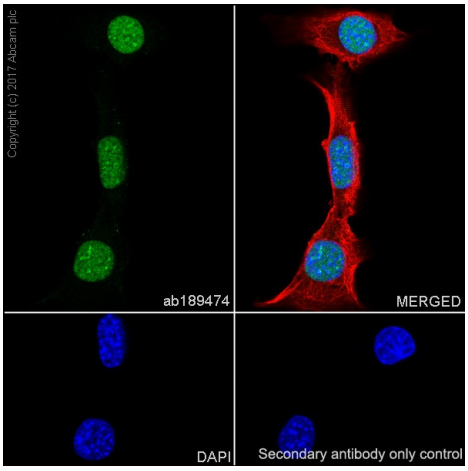


Flow Cytometry - Anti-APE1 antibody [EPR18378-45] - BSA and Azide free (ab227062)

Flow cytometric analysis of 4% pasraformaldehyde-fixed, 90% methanol-permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cell line labeling APE1 with ab189474 at 1/500 dilution (red) compared with a Isotype control details (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue).

Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077), at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab189474).



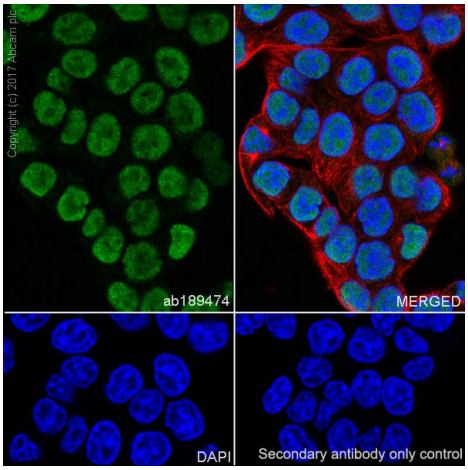
Immunocytochemistry/ Immunofluorescence - Anti-APE1 antibody [EPR18378-45] - BSA and Azide free (ab227062)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cells labeling APE1 with ab189474 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on NIH/3T3 cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution.

-ve control : PBS, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab189474).



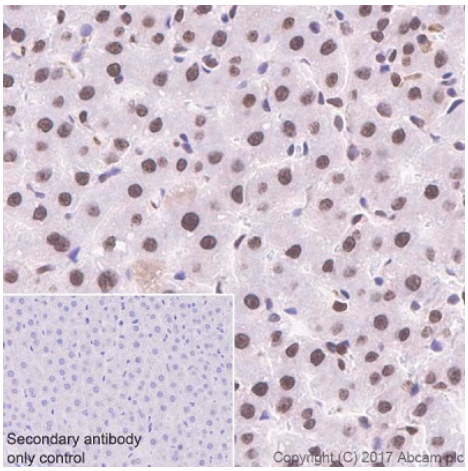
Immunocytochemistry/ Immunofluorescence - Anti-APE1 antibody [EPR18378-45] - BSA and Azide free (ab227062)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT 116 (human colorectal carcinoma cell line) cells labeling APE1 with ab189474 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HCT 116 cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution.

-ve control : PBS, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab189474).



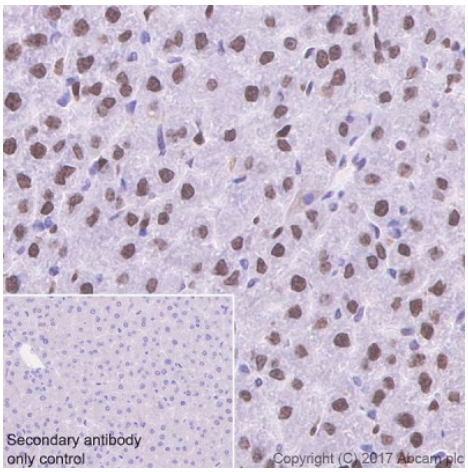
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [EPR18378-45] - BSA and Azide free (ab227062)

Immunohistochemical analysis of paraffin-embedded rat liver tissue labeling APE1 with ab189474 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Mainly nuclear staining on hepatocytes of rat liver (PMID: 10643898) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab189474).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [EPR18378-45] - BSA and Azide free (ab227062)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling APE1 with ab189474 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Immunohistochemical analysis of paraffin-embedded human ovarian cancer tissue labeling APE1 with ab189474 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining on tumor cells of human ovarian carcinoma (PMID: 20087352) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

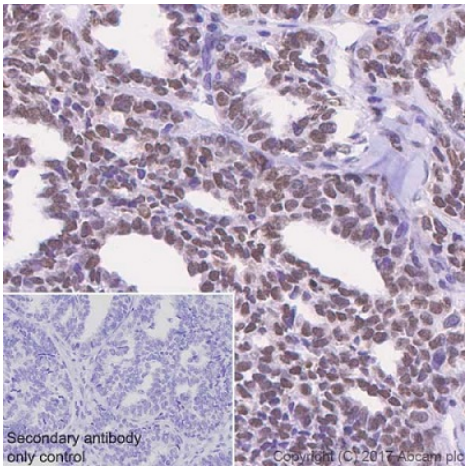
is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (ab189474).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [EPR18378-45] - BSA and Azide free (ab227062)

Immunohistochemical analysis of paraffin-embedded human ovarian cancer tissue labeling APE1 with ab189474 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining on tumor cells of human ovarian carcinoma (PMID: 20087352) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab189474).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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