abcam

Product datasheet

Anti-APE1 antibody [EPR4022] ab92744

KO 評価済 リコンビナント RabMAb

7 References 画像数 10

製品の概要

製品名	Anti-APE1 antibody [EPR4022]
製品の詳細	Rabbit monoclonal [EPR4022] to APE1
由来種	Rabbit
アプリケーション	**適用あり:** WB, IHC-P **適用なし:** Flow Cyt,ICC/IF or IP
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human APE1 aa 1-100 (N terminal). The exact sequence is proprietary.
ポジティブ・コントロール	WB: Wild-type HAP1, K562, HepG2, HEK293, Raji , C6, PC-12, NIH3T3 and Raw264.7 whole cell lysates; rat brain and spleen lysates; mouse brain, heart, kidney and spleen tissue lysates. IHC-P: Human tonsil, human kidney, human liver, mouse liver, and rat spleen tissues.
特記事項	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® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR4022

アイソタイプ IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab92744の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		1/10000 - 1/50000. Predicted molecular weight: 36 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

追加情報 Is unsuitable for Flow Cyt,ICC/IF or IP.

ターゲット情報

機能 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.

配列類似性 Belongs to the DNA repair enzymes AP/ExoA family.

ドメイン 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.

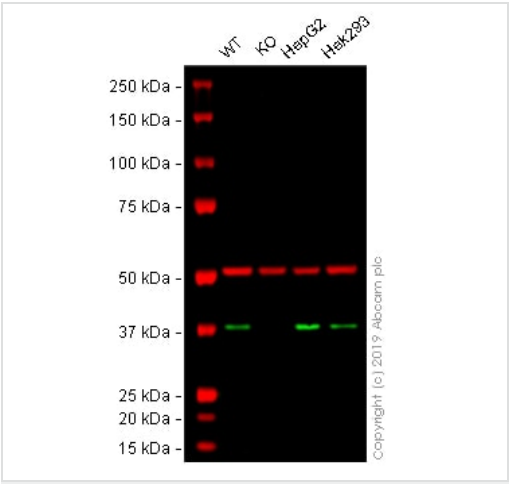
翻訳後修飾

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.

細胞内局在

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.

画像



Western blot - Anti-APE1 antibody [EPR4022] (ab92744)

All lanes : Anti-APE1 antibody [EPR4022] (ab92744) at 1/10000 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

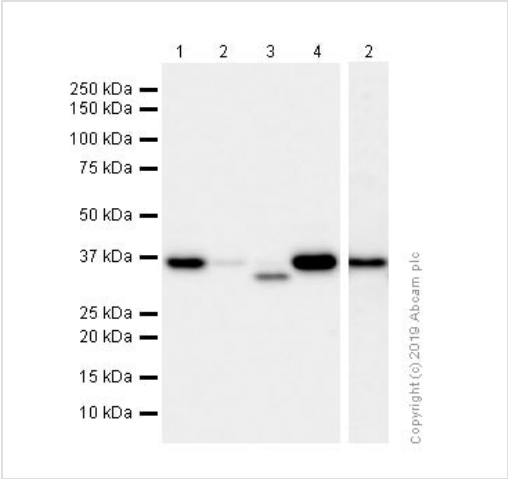
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 36 kDa
Observed band size: 37 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab92744 observed at 37 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab92744 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 ab92744 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 10000 dilution and a 1 in 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 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-APE1 antibody [EPR4022] (ab92744)

All lanes : Anti-APE1 antibody [EPR4022] (ab92744) at 0.51 µg/ml (purified)

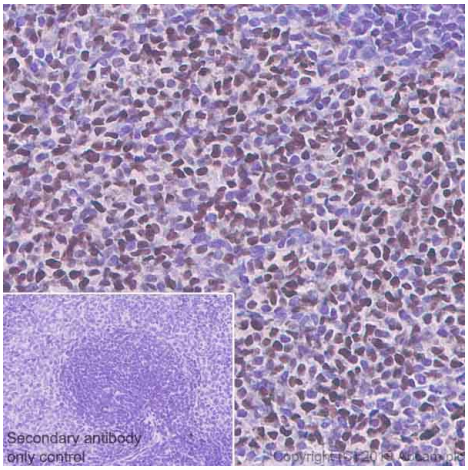
Lane 1 : Mouse brain lysate
Lane 2 : Mouse heart lysate
Lane 3 : Mouse kidney lysate
Lane 4 : Mouse spleen lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 0.05 µg/ml

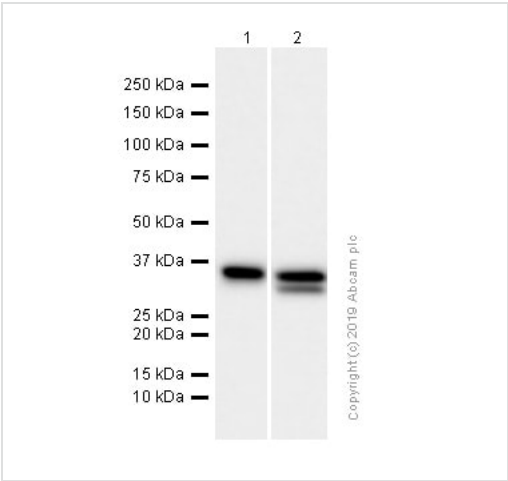
Predicted band size: 36 kDa
Observed band size: 36 kDa

Blocking/diluting buffer and concentration:
5% NFDM /TBST 5% NFDM /TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [EPR4022] (ab92744)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat spleen tissue sections labeling APE1 with purified ab92744 at 1/4000 dilution (0.12 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-APE1 antibody [EPR4022] (ab92744)

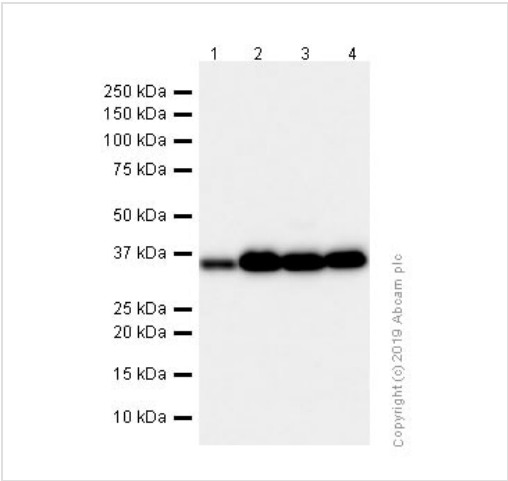
All lanes : Anti-APE1 antibody [EPR4022] (ab92744) at 1/20000 dilution (Purified)

Lane 1 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates
Lane 2 : K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates

Lysates/proteins at 15 µg per lane.

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

Predicted band size: 36 kDa
Observed band size: 36 kDa



Western blot - Anti-APE1 antibody [EPR4022] (ab92744)

All lanes : Anti-APE1 antibody [EPR4022] (ab92744) at 0.51 µg (purified)

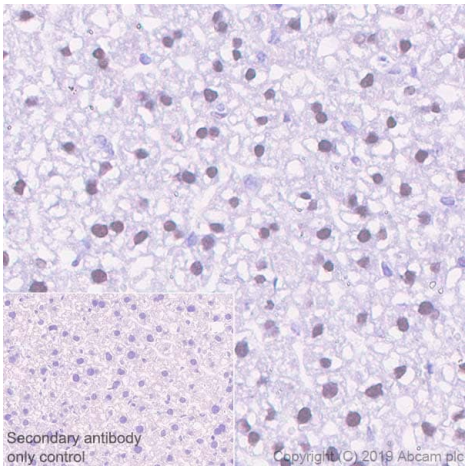
Lane 1 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 2 : Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate
Lane 4 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 0.05 µg/ml

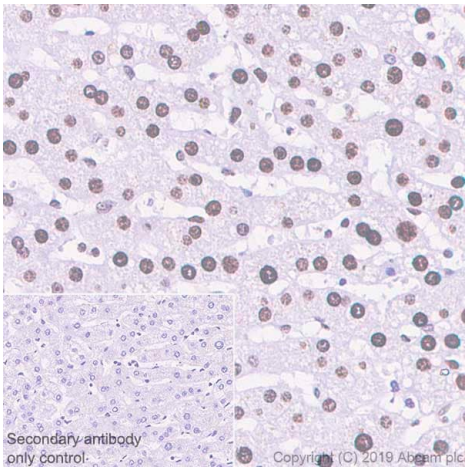
Predicted band size: 36 kDa

Blocking/diluting buffer and concentration:
5% NFDM /TBST 5% NFDM /TBST



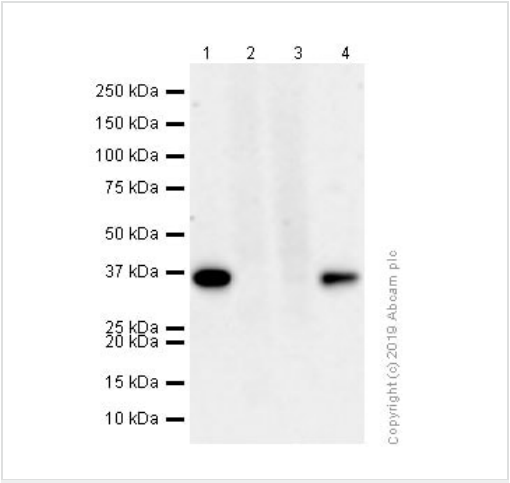
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [EPR4022] (ab92744)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling APE1 with purified ab92744 at 1/4000 dilution (0.12 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [EPR4022] (ab92744)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue sections labeling APE1 with purified ab92744 at 1/4000 dilution (0.12 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



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All lanes : Anti-APE1 antibody [EPR4022] (ab92744) at 0.51 µg/ml (purified)

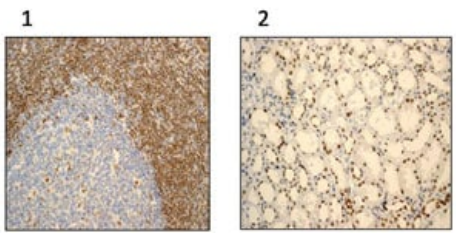
Lane 1 : Rat brain lysate
Lane 2 : Rat heart lysate
Lane 3 : Rat liver lysate
Lane 4 : Rat spleen lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 0.05 µg/ml

Predicted band size: 36 kDa
Observed band size: 36 kDa

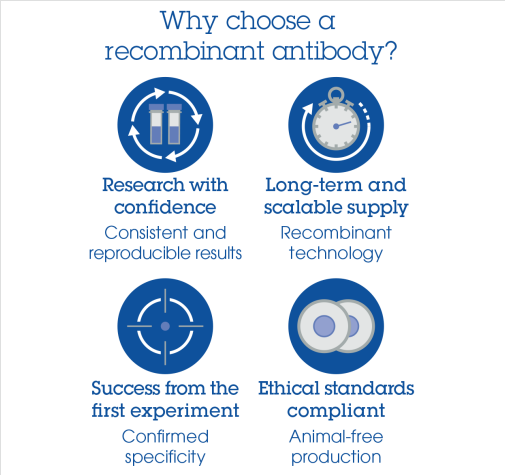
Blocking/diluting buffer and concentration:
5% NFDM /TBST 5% NFDM /TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [EPR4022] (ab92744)

Unpurified ab92744, at a 1/100 dilution, staining APE1 in formalin fixed, paraffin embedded (1) Human tonsil tissue and (2)Human kidney tissue by Immunohistochemistry. Detection: DAB staining.

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



Anti-APE1 antibody [EPR4022] (ab92744)

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