abcam

Product datasheet

# Anti-APE1 antibody [EPR18378-45] - ChIP Grade ab189474

敲除验证 重组 RabMAb

4 References 12 图像

## 概述

| | |
|---|---|
| **产品名称** | Anti-APE1抗体[EPR18378-45] - ChIP Grade |
| **描述** | 兔单克隆抗体[EPR18378-45] to APE1 - ChIP Grade |
| **宿主** | Rabbit |
| **经测试应用** | **适用于:** Flow Cyt (Intra), IHC-P, ICC/IF, WB, ChIP |
| **种属反应性** | **与反应:** Mouse, Rat, Human |
| **免疫原** | Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. |
| **阳性对照** | WB: Human fetal brain, fetal heat, fetal kidney and fetal spleen tissue lysate; mouse brain, heart and liver tissue lysate; rat brain, liver and spleen tissue lysate; HCT 116, HeLa, NIH/3T3, HEK293, HepG2, wild-type HAP1, and C6 whole cell lysates. IHC-P: Human ovarian carcinoma tissue; mouse liver tissue; rat liver tissue. ICC/IF: HCT 116 and NIH/3T3 cells. Flow Cyt (intra): NIH/3T3 cells. ChIP: HCT 116 cells. |
| **常规说明** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. |

## 性能

| | |
|---|---|
| **形式** | Liquid |
| **存放说明** | 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. |
| **存储溶液** | pH: 7.2<br>Preservative: 0.01% Sodium azide<br>Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS |
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |

| | |
|---|---|
| **克隆编号** | EPR18378-45 |
| **同种型** | IgG |

## 应用

**The Abpromise guarantee** **Abpromise™承诺保证使用ab189474于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

| 应用 | Ab评论 | 说明 |
|---|---|---|
| **Flow Cyt (Intra)** | | 1/500. |
| **IHC-P** | | 1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **ICC/IF** | | 1/250. |
| **WB** | | 1/1000. Predicted molecular weight: 35 kDa. |
| **ChIP** | | Use 5 µg for 25 µg of chromatin. |

## 靶标

**功能** 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.

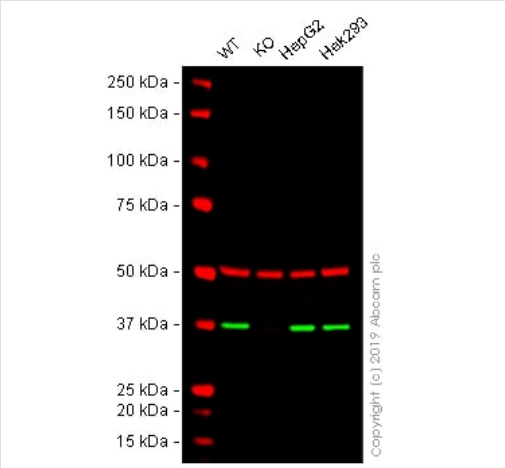
**序列相似性** Belongs to the DNA repair enzymes AP/ExoA family.

**结构域** 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.

**翻译后修饰** 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.

**细胞定位** 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.

## 图片




Western blot - Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474)

**All lanes :** Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474) at 1/1000 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

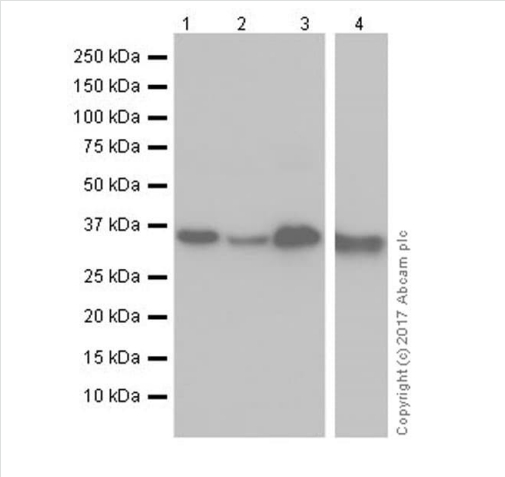
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 35 kDa
**Observed band size:** 37 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab189474 observed at 37 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab189474 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 ab189474 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 1000 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 [EPR18378-45] - ChIP Grade (ab189474)

**All lanes :** Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474) at 1/1000 dilution

**Lane 1 :** Human fetal brain lysate
**Lane 2 :** Human fetal heart lysate
**Lane 3 :** Human fetal kidney lysate
**Lane 4 :** Human fetal spleen lysate

Lysates/proteins at 10 µg per lane.

**Secondary**
**All lanes :** VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/2000 dilution

Developed using the ECL technique.

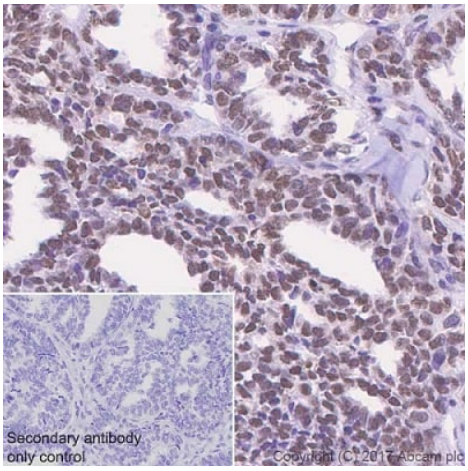
**Predicted band size:** 35 kDa
**Observed band size:** 35 kDa

Blocking and dilution buffer: 5% NFDM/TBST

Exposure times:

Lanes 1-3: 1 second

Lane 4: 4 seconds

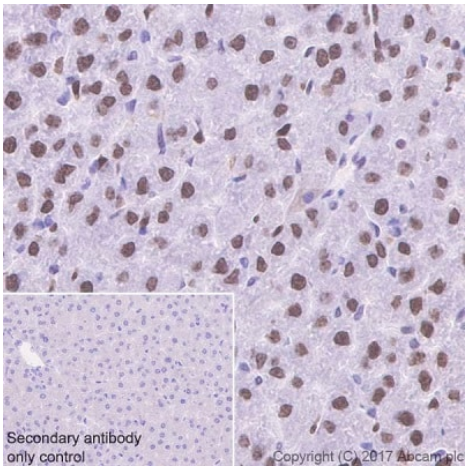



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474)

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.

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] - ChIP Grade (ab189474)

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.

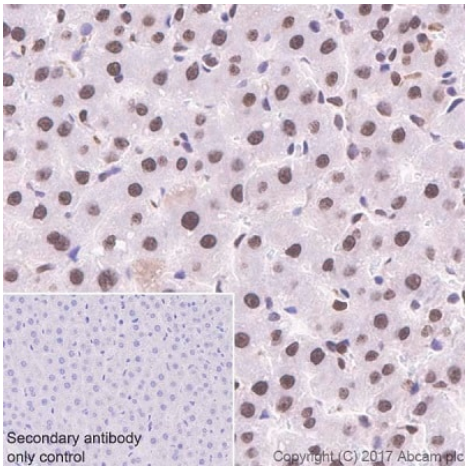
Immunohistochemical analysis of paraffin-embedded human ovarian cancer tissue labeling APE1 with ab189474 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining on tumor cells of human ovarian carcinoma (PMID: 20087352) is observed. Counter stained with hematoxylin.

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.

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

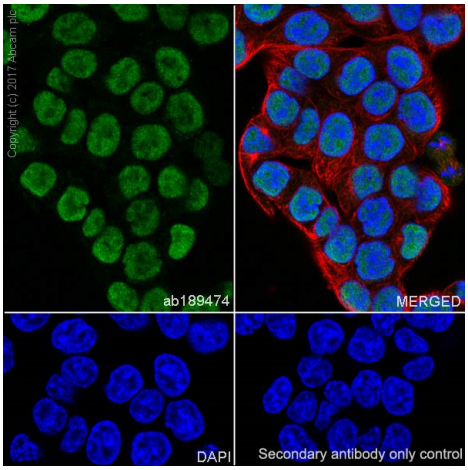



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474)

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.

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

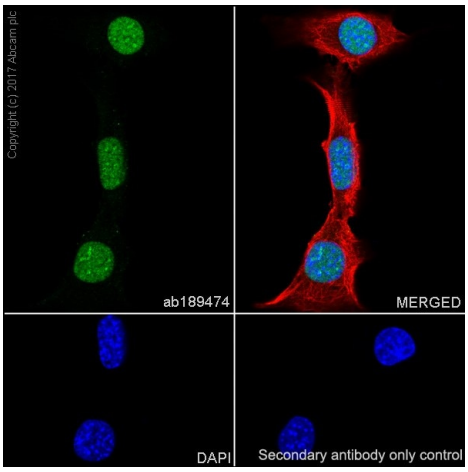



Immunocytochemistry/ Immunofluorescence - Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474)

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

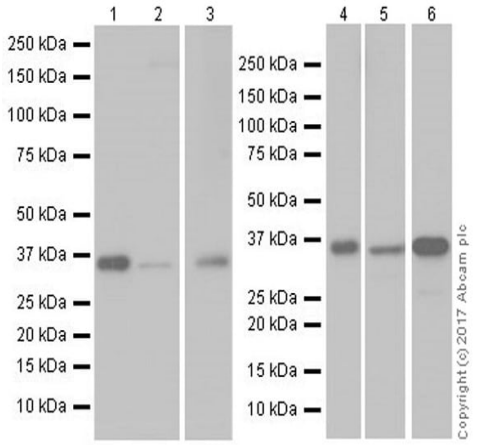



Immunocytochemistry/ Immunofluorescence - Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474)

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




Western blot - Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474)

**All lanes :** Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474) at 1/1000 dilution

**Lane 1 :** Mouse brain lysate
**Lane 2 :** Mouse heart lysate
**Lane 3 :** Mouse liver lysate
**Lane 4 :** Rat brain lysate
**Lane 5 :** Rat liver lysate
**Lane 6 :** Rat spleen lysate

Lysates/proteins at 10 µg per lane.

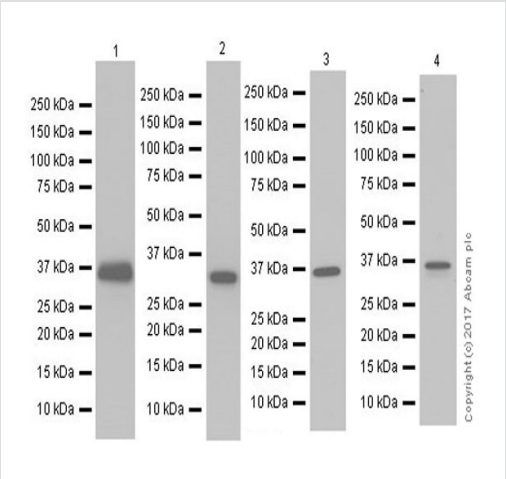
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

**Predicted band size:** 35 kDa
**Observed band size:** 35 kDa

Blocking and dilution buffer: 5% NFDM/TBST

Exposure times:




Western blot - Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474)

Lanes 1-5: 8 seconds

Lane 6: 4 seconds

**All lanes :** Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474) at 1/5000 dilution

**Lane 1 :** HCT 116 (human colorectal carcinoma cell line) whole cell lysate
**Lane 2 :** HeLa(human epithelial cell line from cervix adenocarcinoma) whole cell lysate
**Lane 3 :** NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate
**Lane 4 :** C6 (rat brain glioma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

**Predicted band size:** 35 kDa
**Observed band size:** 35 kDa

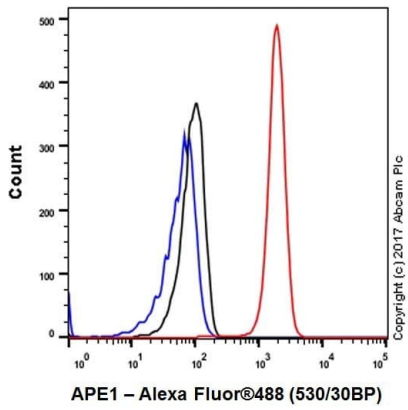
Blocking and dilution buffer: 5% NFDM/TBST

Exposure times:

Lanes 1 & 2: 1 second

Lane 3: 5 seconds

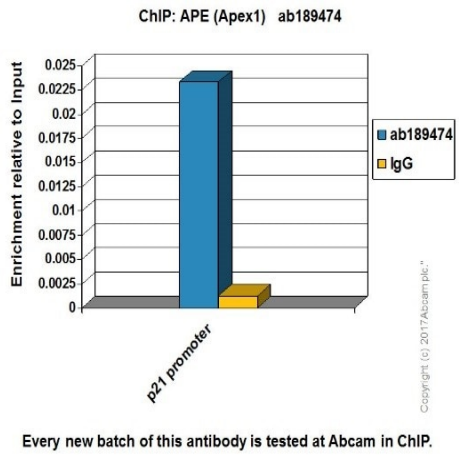
Lane 4: 3 seconds




Flow Cytometry (Intracellular) - Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474)

Intracellular flow cytometric analysis of4% 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).

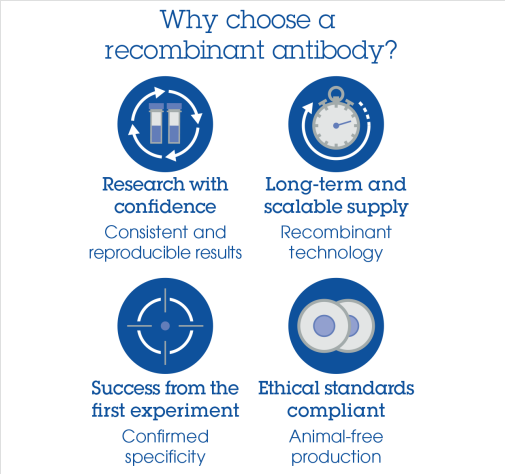
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**), at 1/2000 dilution was used as the secondary antibody.




ChIP - Anti-APE1 antibody [EPR18378-45] - ChIP Grade (ab189474)

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).




Anti-APE1 antibody [EPR18378-45] - ChIP Grade

(ab189474)

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