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Product datasheet

Mouse APE ELISA Kit ab207616

Recombinant SimpleStep ELISA

1 References 8 Images

Overview

Product name Mouse APE ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
C2C12	8			3.1%

Inter-assay

Sample	n	Mean	SD	CV%
C2C12	3			8.8%

Sample type Cell culture supernatant, Cell culture extracts, Tissue Extracts

Assay type Sandwich (quantitative)

Sensitivity 28.8 pg/ml

Range 46.88 pg/ml - 3000 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture media	100	98% - 104%

Assay time 1h 30m

Assay duration One step assay

Species reactivity **Reacts with:** Mouse

Product overview Mouse APE ELISA Kit (ab207616) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of APE protein in cell culture extracts, cell culture supernatant, and tissue extracts. It uses our proprietary SimpleStep ELISA® technology. Quantitate Mouse APE with 28.8 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is

recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (**ab203359**) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

Notes

Mouse APE is a multifunctional protein that binds DNA and RNA and plays a central role in the cellular response to oxidative stress. The two major activities of APE are DNA repair and redox regulation of transcription factors. APE functions as an apurinic/apyrimidinic (AP) endodeoxyribonuclease in the DNA base excision repair (BER) pathway of DNA lesions induced by oxidative and alkylating agents. The C-terminal region of APE is responsible for base excision repair activities. Notably, this repair activity is suppressed by phosphorylation. The N-terminal region of APE may regulate the DNA binding capabilities of a variety of transcription factors through redox stimulation. Mouse APE has 98% and 94% identity to rat and human APE, respectively.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Microplate (12 x 8 well strips)

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Mouse APE Capture Antibody	1 x 600µl
10X Mouse APE Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 4BI	1 x 6ml
Mouse APE Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit

Components	1 x 96 tests
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

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

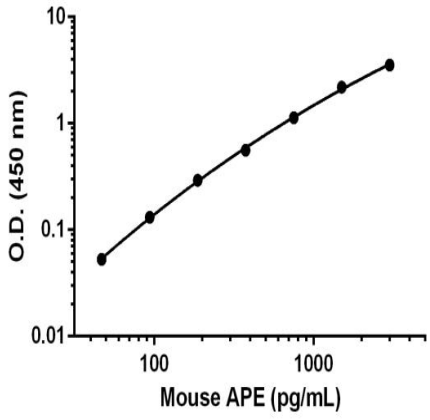
Phosphorylated. Phosphorylation by kinase PKC or casein kinase CK2 results in enhanced redox activity that stimulates binding of the FOS/JUN AP-1 complex to its cognate binding site. AP-endodeoxyribonuclease activity is not affected by CK2-mediated phosphorylation.
Phosphorylation of Thr-233 by CDK5 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

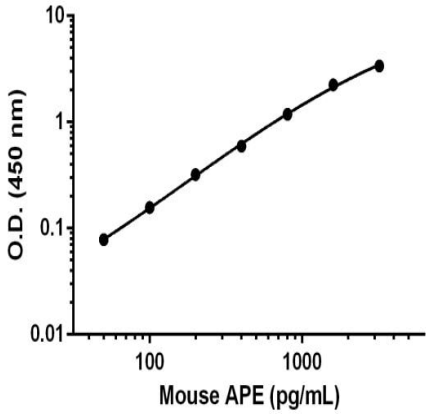
Mitochondrion. The cleaved APEX2 is only detected in mitochondria (By similarity). Translocation from the cytoplasm to the mitochondria is mediated by ROS signaling and cleavage mediated by granzyme A. Tom20-dependent translocated mitochondrial APEX1 level is significantly increased after genotoxic stress 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



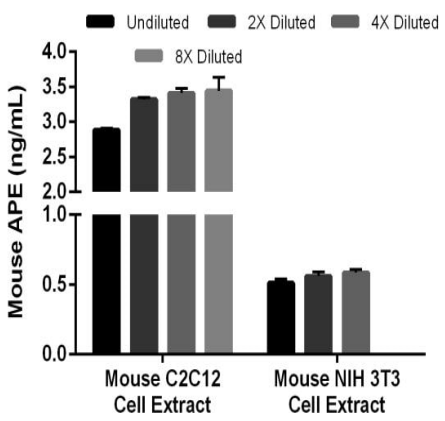
Example of mouse APE standard curve in Sample Diluent NS.

Background-subtracted data values (mean +/- SD) are graphed.



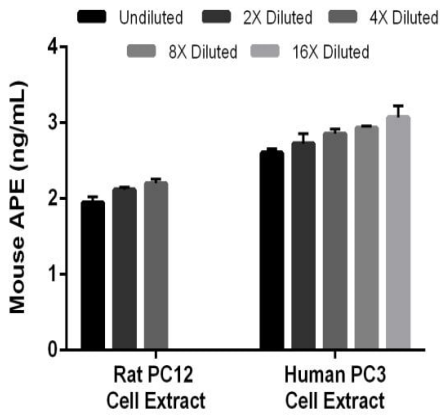
Example of mouse APE standard curve in 1X Cell Extraction Buffer PTR.

Background-subtracted data values (mean +/- SD) are graphed.



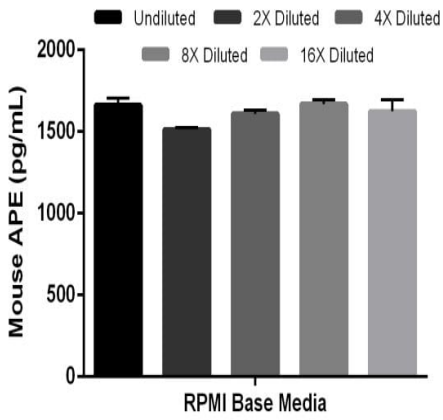
Interpolated concentrations of native APE in mouse C2C12 cell extract and NIH 3T3 cell extract based on 150 µg/mL and 3.13 µg/mL extract loads, respectively.

The concentrations of APE were measured in duplicate and interpolated from the APE standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean APE concentration was determined to be 3.26 ng/mL in C2C12 cell extract and 0.55 ng/mL in NIH 3T3 cell extract.



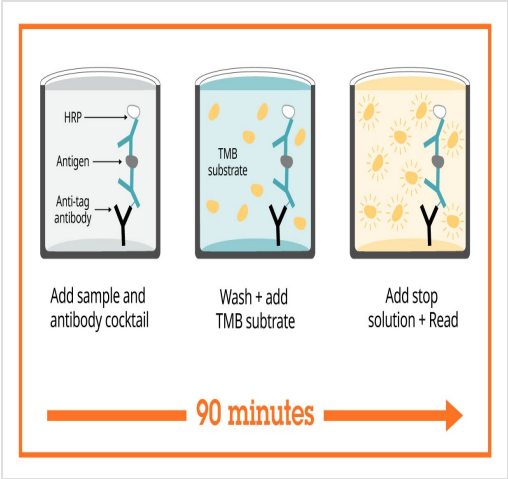
Interpolated concentrations of native APE in rat PC12 cell extract and mouse PC3 cell extract based on 12.5 µg/mL and 100 µg/mL extract loads, respectively.

The concentrations of APE were measured in duplicate and interpolated from the APE standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean APE concentration was determined to be 2.09 ng/mL in rat PC12 cell extract and 2.84 ng/mL in mouse PC3 cell extract.



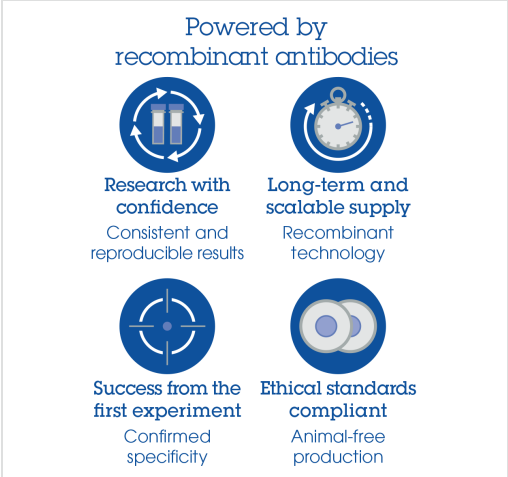
Interpolated concentrations of spiked APE in RPMI Base Media.

The concentrations of APE were measured in duplicates, interpolated from the APE standard curve and corrected for sample dilution. Undiluted samples are as follows: RPMI Base Media 10%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean APE concentration was determined to be 1,616 pg/mL in RPMI Base Media 10%.



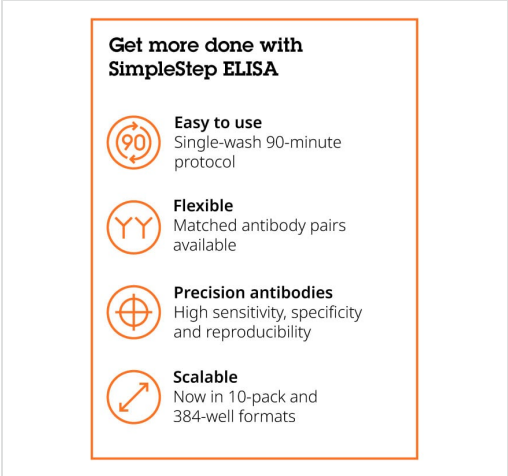
Sandwich ELISA - Mouse APE ELISA Kit (ab207616)

SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



Sandwich ELISA - Mouse APE ELISA Kit (ab207616)

To learn more about the advantages of recombinant antibodies see **here**.



Sandwich ELISA - Mouse APE ELISA Kit (ab207616)

To learn more about the advantages of SimpleStep ELISA® kits see **here**.

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