

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human APE1 Polyclonal, IgG***Cat ID# - PA-RP 000009*Identifyn® Secondary Antibody**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey anti-Rabbit, IgG (H + L)***Cat ID# - SA 000060*

APE1 (AP endonuclease 1), also known as APEX1 or Ref-1, is a pivotal enzyme in the base excision repair (BER) pathway. This pathway, which is of paramount importance for repairing single-strand breaks (SSBs) and other forms of DNA damage, relies heavily on APE1.

APE1 in Base Excision Repair (BER)

Base excision repair (BER) is a significant pathway for correcting small base lesions, such as oxidized or deaminated bases, which can result in single-strand breaks if not repaired properly. APE1 is an essential component of the BER pathway and is specifically involved in the following:

AP Site Recognition and Cleavage: APE1's primary function is to recognize and cleave abasic sites, which are locations in DNA where the base has been removed but the phosphate backbone remains intact. These abasic sites, also known as apurinic/apyrimidinic (AP) sites, can arise from spontaneous base loss or from the action of DNA glycosylases.

Endonuclease Activity: After recognizing an AP site, APE1 cleaves the DNA backbone, generating a single-strand break with a 3'-hydroxyl (3'-OH) end and a 5'-deoxyribose phosphate (5'-dRP) end. This cleavage creates a suitable entry point for further repair steps in the BER pathway.

APE1 and Single-Strand Break Repair

Once APE1 creates a single-strand break during base excision repair, the following steps occur to complete the repair process:

Gap Filling and Strand Sealing: DNA polymerase β (Pol β) extends the 3'-OH end by adding the appropriate nucleotide(s) to fill the gap. DNA ligase III, often in complex with XRCC1 (X-ray Repair Cross-Complementing Protein 1), seals the break by forming a phosphodiester bond at the 5'-dRP end.

Interaction with Other Proteins: APE1 interacts with multiple proteins involved in SSB and BER, including XRCC1 and DNA ligase III. These interactions help coordinate the repair process and ensure efficiency.

Additional Functions of APE1

Besides its crucial role in DNA repair, APE1 has other essential functions:

Redox Activity: APE1 acts as a redox factor, regulating the activity of various transcription factors by maintaining their redox state. This role is critical for controlling gene expression related to stress response, growth, and survival.

Therapeutic Implications: Given its central role in DNA repair, APE1 is a potential therapeutic target in cancer therapy. Modulating APE1 activity may influence DNA repair capacity and cellular response to DNA damage.

APE1's role in single-strand break repair is crucial within the base excision repair pathway. By recognizing and processing AP sites, APE1 initiates the repair process, successfully restoring DNA integrity. Its additional functions and therapeutic implications make it a protein of considerable interest in basic research and clinical applications.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20 °C methanol and blocked with [Thermo Fisher Fish Serum Blocker \(cat#37527X3\)](#). The primary antibody, Rabbit anti-Human APE1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI \(cat#P36962\)](#).

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.110 µm X 0.110 µm

Image Size (Pixels) = 1021 X 881

Image Size (Scaled) = 111.82 µm X 96.49 µm

Bit Depth = 14 Bit

Image Center Position = X: 0.00 µm, Y: 0.00 µm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = 50 Cy 5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715

Contrast Method = Fluorescence

Light Source = X-Cite Xylis Lamp Intensity @ 20.00%

Exposure Time = 200 ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.4

Imaging Device = Axiocam 705

Camera Adapter = 1X

Depth of Focus = 1.03 µm

Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI

Beam Splitter = 395

Filter Excitation Wavelength = 335 - 383

Filter Emission Wavelength = 420 - 470

Contrast Method = Fluorescence

Light Source = X-Cite Xylis Lamp Intensity @ 10.00%

Exposure Time = 30 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam 807 Mono
 Camera Adapter = 1X
 Depth of Focus = 0.72 μm
 Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey anti-Rabbit, IgG (H + L) secondary antibody. The middle panel shows DAPI staining, and the right panel shows the two-channel merged image.

Colocalization View - Panel B

Horizontal Axis - AF647, Threshold 183, Range Auto
 Vertical Axis - DAPI, Threshold 94, Range Auto
 Image Measurement = Entire Field

Demonstrates recruitment of APE1 to the nucleus in response to etoposide-induced DNA damage. Colocalization of DAPI and APE1 indicates that APE1 is localized within the nuclear compartment.

Summary

The left panel shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), demonstrating a strong nuclear-associated fluorescence signal following etoposide-induced DNA damage. The middle panel shows DAPI nuclear counterstaining, while the right panel presents the merged image demonstrating predominant nuclear localization of APE1 with minimal background signal under widefield fluorescence microscopy conditions.

APE1 is a critical component of the base excision repair (BER) pathway and is responsible for processing apurinic/apyrimidinic (AP) sites generated during DNA damage repair. Following etoposide treatment, increased nuclear localization of APE1 is consistent with activation of DNA repair signaling and recruitment of BER-associated repair machinery.

The dataset demonstrates strong compatibility between Rabbit anti-Human APE1 Polyclonal, IgG and Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), supporting robust fluorescence signal generation, low-background staining characteristics, and preservation of biologically consistent nuclear localization patterns under standard widefield fluorescence microscopy acquisition conditions.

Image Correction

NONE

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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