

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human APE1 Polyclonal, IgG***Cat ID# - PA-RP 000009***Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)***Cat ID# - PA-MM 000001*Identifyn® Secondary Antibody**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000060***Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific***Cat ID# - SA 000034*

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 methanol at -20 °C 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.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962](#).

Microscope

Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 78 of 171, Panel A
Z-Stack = 171 Slices (6.0 µm)

Channels = 2
 Scaling (Per Pixel) = 0.035 µm X 0.035 µm X 0.030 µm
 Image Size (Pixels) = 722 X 943
 Image Size (Scaled) = 25.49 µm X 33.29 µm
 Bit Depth = 16 Bit
 Image Center Position = X: 91.65 mm, Y: 49.86 mm
 Stage Position = X: 91.65 mm, Y: 49.86 mm
 ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B
Z-Stack = 171 Slices (6.0 µm)

Channels = 2
 Scaling (Per Pixel) = 0.035 µm X 0.035 µm X 0.030 µm

Image Size (Pixels) = 228 X 241
Image Size (Scaled) = 8.05 μm X 8.51 μm
Bit Depth = 16 Bit
Image Center Position = X: 91.65 mm, Y: 49.86 mm
Stage Position = X: 91.65 mm, Y: 49.86 mm
ROI Center Offset = X: 0.00 μm , Y: 0.00 μm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 280 μm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = **640 nm @ 1.00%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.62 μs
Frame Time = 8.36 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 700
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.9 (3D, Auto)

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 243 μm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = **561 nm @ 2.40%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.62 μs
Frame Time = 8.36 s
LSM Scan Speed = 7
Scan Direction = Bidirectional

Line Step = 1
 Averaging = 4
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 560 - 600
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = SuperResolution 8.8 (3D, Auto)

2-Dimensional View- Panel A

Shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

3D Volume Projection = Precise
 Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Shown from the same product data set to demonstrate the resolution limitations of Airyscan while also demonstrating improved localization and structural detail relative to conventional confocal imaging.

Summary

Panel A shows a single optical section (Z-plane 78/171) depicting APE1 and PARP1 distribution following etoposide-induced DNA damage. APE1 (rabbit polyclonal) is detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody (red), and PARP1 (monoclonal) is detected with Identifyn® SRM Alexa Fluor™ 555 goat anti-mouse secondary antibody, pseudocolored white for visual contrast. Panel B provides a three-dimensional volume reconstruction of the ROI from Panel A, highlighting the spatial organization of APE1 and PARP1 within the imaged volume.

At the diffraction-limited scale, the signals appear partially overlapping; however, this likely reflects limited resolution rather than direct molecular association. PARP1 functions as an early sensor of DNA strand breaks, rapidly binding damaged DNA and initiating

repair signaling through PARylation, whereas APE1 (~37 kDa) acts downstream in the base excision repair pathway to process abasic sites. Their distributions are therefore consistent with coordinated, but mechanistically distinct, roles within DNA damage response regions.

Conventional and Airyscan imaging remain resolution-limited and may conflate closely apposed but distinct structures. Panel C shows STORM imaging of APE1 alone, revealing refined structural detail and sub-diffraction organization not resolved in lower-resolution modalities. These observations indicate that the apparent signal patterns in Panels A and B reflect broader spatial organization, while finer nanoscale structure remains unresolved without higher-resolution approaches.

Image Correction

The PARP1 channel (555) was pseudocolored white to enhance visual contrast between signals.

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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