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

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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 69 of 141, Panels A, B, and C*
Z-Stack = 141 Slices (6.0 µm)

Channels = 3
Scaling (Per Pixel) = 0.071 µm X 0.071 µm X 0.030 µm
Image Size (Pixels) = 397 X 457
Image Size (Scaled) = 28.02 µm X 32.26 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.46 mm, Y: 46.80 mm
Stage Position = X: 86.64 mm, Y: 46.80 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 56 µm

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LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = **640 nm @ 0.80%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.59 µs
Frame Time = 2.22 s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 630 - 700
Imaging Device = GaAsP-Pmt2
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 49 µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = **561 nm @ 0.20%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.59 µs
Frame Time = 2.22 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 557 - 603
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

DAPI -

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Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 37 µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = **405 nm @ 1.50%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.59 µs
Frame Time = 2.22 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410 - 470
Imaging Device = GaAsP-Pmt1
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

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 top-right panel shows 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. The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

Profile View Display - *Panels B & C*

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1
Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 30.9), Y (Min 0 and Max 12908)

This panel quantifies APE1 foci, representing diffraction-limited signal aggregates, following etoposide-induced DNA damage. Line profile analysis reveals well-defined intensity peaks and spatial confinement, supporting localized enrichment of APE1 at damage-associated regions.

Maximum Peak - 12,293.00 A.U.

Zen Acquisition Info Tab - *Panel D*

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Shown are the acquisition parameters for the 647 channel (APE1) and 555 channel (PARP1), collected using the 640 nm laser at 0.80% power and the 561 nm laser at 0.20% power, respectively. Scan zoom, scan speed, and line-averaging settings are provided to ensure the transparency and reproducibility of line-profile measurements.

Summary

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), demonstrating discrete nuclear-associated APE1 fluorescence following etoposide-induced DNA damage. The top-right panel shows 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, highlighting PARP1 distribution within the nuclear compartment. The lower-left panel shows DAPI nuclear counterstaining, while the bottom-right panel presents the three-channel merged image demonstrating overlapping nuclear-associated localization patterns between APE1 and PARP1 under confocal fluorescence microscopy conditions.

APE1 is a critical component of the base excision repair (BER) pathway and functions downstream of DNA damage recognition to process abasic intermediates generated during DNA repair. PARP1 serves as an early sensor of DNA strand breaks and is rapidly recruited to sites of genomic damage following etoposide treatment. The observed spatial proximity between APE1 and PARP1 is consistent with coordinated engagement of DNA damage response and BER-associated repair pathways within the nuclear compartment.

Line-profile analysis demonstrates discrete APE1 fluorescence intensity peaks and localized signal confinement, supporting the formation of resolution-limited repair-associated foci following DNA damage induction. The dataset further demonstrates strong compatibility between Rabbit anti-Human APE1 Polyclonal, IgG and Identifyn® SRM Alexa Fluor™ secondary antibody systems under confocal acquisition conditions, supporting robust fluorescence signal generation, low-background imaging characteristics, and preservation of biologically consistent nuclear localization patterns.

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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