

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 DNA repair, 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.

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 Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 64 of 147, Panel A
Z-Stack = 147 Slices (4.38 µm)

Channels = 2
 Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
 Image Size (Pixels) = 1550 X 1220
 Image Size (Scaled) = 35.72 µm X 25.76 µm
 Bit Depth = 16 Bit
 Image Center Position = X: 63.80 mm, Y: 37.58 mm
 Stage Position = X: 63.80 mm, Y: 37.58 mm
 ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - 3D Volume Projection, Panel B
Z-Stack = 147 Slices (4.38 µm)

Channels = 2
 Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
 Image Size (Pixels) = 251 X 245

Image Size (Scaled) = 5.30 μm X 5.17 μm
Bit Depth = 16 Bit
Image Center Position = X: 63.80 mm, Y: 37.58 mm
Stage Position = X: 63.80 mm, Y: 37.58 mm
ROI Center Offset = X: 0.00 μm , Y: 0.00 μm

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 1.13 μm
Binning Mode = 2,2
Laser Wavelength = 640 nm @ 5.0%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 μm grating

555 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 555
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 0.92 μm
Binning Mode = 2,2
Laser Wavelength = 561 nm @ 4.0%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 μm grating

Post Processing - SIM Processing

Processing Dimension = 3D
 Result Sampling = 4
 Process Sampling = N/A
 Channels = 2
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 9.9.77 (647), 11.2499 (555)
 Fitting = Fast Fit
 Normalization = 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 100%, 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 dataset to demonstrate the resolution limitations of Lattice SIM while also highlighting its improved localization and structural detail relative to Airyscan imaging. The observed signal distribution is consistent with the corresponding Airyscan dataset; however, the structures are more resolution-limited and less spatially refined than those observed with single-molecule localization microscopy by STORM.

Summary

Using structured illumination microscopy (SIM), Panel B shows the organization of APE1 and PARP1 following etoposide-induced DNA damage. Structures that appear as single foci under confocal and Airyscan imaging are partially resolved into spatially distinct features by SIM, revealing underlying heterogeneity within APE1- and PARP1-associated aggregates. However, despite this improvement, the observed structural detail remains constrained by the SIM's effective resolution.

In Panel C, single-molecule STORM imaging of APE1 alone provides substantially greater structural refinement, resolving these aggregates into smaller, discrete substructures at

the sub-diffraction scale. While certain spatial patterns observed in Panel B show reproducible similarities to those seen by STORM, these correspondences remain qualitative and should be interpreted with caution. The features visualized by SIM appear enlarged and lack the fine architectural detail revealed by STORM.

Importantly, APE1 (~37 kDa) and PARP1 (~113 kDa) are both small relative to the apparent dimensions of the SIM-resolved features, which substantially exceed the expected molecular scale. This establishes a direct physical constraint, indicating that the observed structures are dominated by the diffraction-limited point spread function and labeling geometry (including antibody size and fluorophore displacement), rather than representing true molecular dimensions. As such, SIM captures resolution-limited aggregates, whereas STORM begins to resolve underlying nanoscale organization.

Collectively, these data demonstrate that the organization of APE1 and PARP1 in response to DNA damage remains incompletely resolved by SIM, with significant substructure below its resolving capability.

Image Correction

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

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

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