

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.

Method

Plus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation 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 \(cat# P36962\)](#).

Minus Damage -

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 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 71 of 141, Panel A, from the Confocal Dataset - Plus Damage

Z-Stack = 141 Slices (4.2 μ m)

Channels = 2

Scaling (Per Pixel) = 0.071 μ m X 0.071 μ m X 0.030 μ m

Image Size (Pixels) = 898 X 898

Image Size (Scaled) = 63.38 μ m X 63.38 μ m

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 μ m, Y: 0.00 μ m

Z Stack - (3D) - 2D at Z-Plane 80 of 181 - Control, Minus Damage

Z-Stack = 181 Slices (5.4 μ m)

Channels = 1

Scaling (Per Pixel) = $0.071\ \mu\text{m} \times 0.071\ \mu\text{m} \times 0.030\ \mu\text{m}$
Image Size (Pixels) = 501 X 524
Image Size (Scaled) = $35.37\ \mu\text{m} \times 37.00\ \mu\text{m}$
Bit Depth = 16 Bit
Image Center Position = X: 73.71 mm, Y: 50.38 mm
Stage Position = X: 73.71 mm, Y: 50.38 mm
ROI Center Offset = X: $0.00\ \mu\text{m}$, Y: $0.00\ \mu\text{m}$

647 - Plus Damage, from Confocal Data Set

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / $56\ \mu\text{m}$
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 = $0.59\ \mu\text{s}$
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

647 - Minus Damage - noting the same laser input power and conditions

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / $56\ \mu\text{m}$
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = **640 nm @ 0.80%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 1.00
Pixel Time = $0.55\ \mu\text{s}$
Frame Time = 3.26 s
LSM Scan Speed = 9
Scan Direction = Bidirectional

Line Step = 1
 Averaging = 8
 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

Profile View Display - *Plus DNA Damage, Top*

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - **X (Min 0.0 and Max 37.60), Y (Min 0.0 and Max 15259)**

This panel measures APE1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately 15K A.U. at its maximum.

Measured at the Z-plane, 71 of 141.

Profile View Display - *Minus DNA Damage, Bottom*

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - **X (Min 0.0 and Max 35.5), Y (Min 0.0 and Max 2322)**

This panel measures APE1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately 2K A.U. at its maximum.

Measured at the Z-plane, 80 of 181.

Histogram Display - *Plus DNA Damage, Top*

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 5000000)

Arithmetic Mean intensity = 1142.219

The histogram was generated from the yellow-boxed region to quantify the overall APE1 response within a defined nuclear area following DNA damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - *Minus DNA Damage, Bottom*

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 200000)

Arithmetic Mean intensity = 895.358

The histogram was generated from the yellow-boxed region to quantify the overall APE1 protein within a defined nuclear area in the absence of DNA damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusions -

In undamaged cells, APE1 exhibits low-intensity, predominantly diffuse nuclear distribution, consistent with its basal role in DNA maintenance. Following etoposide-induced DNA damage, APE1 shows a marked increase in mean intensity and reorganizes into discrete nuclear foci, with aggregate signal elevated by ~12-fold.

This redistribution is consistent with activation of the DNA damage response and recruitment of APE1 to sites of DNA lesions within the base excision repair (BER) pathway. In this context, APE1 functions downstream of initial damage recognition, where PARP1-dependent signaling and PARylation facilitate the assembly of repair complexes. The observed focal accumulation of APE1 is therefore consistent with its localization to PARP1-associated repair microenvironments rather than direct damage sensing.

The increase in signal intensity and organization reflects the concentration of APE1 at sites of repair activity, supporting its enzymatic role in processing abasic (AP) intermediates within damage-associated regions. While these foci represent active repair domains, spatial proximity to PARP1 does not imply direct molecular interaction but rather coordinated engagement within shared repair pathways.

Collectively, these data support a model in which APE1 redistribution following genotoxic stress reflects PARP1-facilitated recruitment to DNA repair sites, reinforcing its role as a downstream effector within the BER machinery.

Image Corrections

None

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.