Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, F(ab')$_2$ (H + L)
Cat ID# - SA 000062

PARP1 is one of the first proteins to respond to single-strand breaks in DNA. It can detect DNA damage because its zinc finger domain binds to the break site.

Recruitment of Repair Machinery

Once PARP1 detects a single-strand break, it activates and synthesizes poly(ADP-ribose) (PAR) chains using NAD+ as a substrate. This process is known as PARylation. The PAR chains serve as a signal to recruit and organize other proteins involved in DNA repair.

Coordination of DNA Repair Proteins

PARylation by PARP1 leads to the recruitment of critical proteins involved in the SSB repair pathway, such as XRCC1, DNA ligase III, and DNA polymerase β. These proteins play specific roles in the repair process, like filling in missing nucleotides and rejoining DNA strands.

Regulation of Chromatin Structure

PARylation by PARP1 can also impact chromatin structure, loosening it to allow better access for repair proteins, which is crucial, as tightly packed chromatin could impede the repair process.

Auto-regulation and Resolution

After coordinating the repair process, PARP1 is removed from the break site by dissociating from the PAR chains or by degradation, ensuring it doesn't persistently signal for repair or interfere with the completion of the process.

Prevention of Unwanted DNA Damage

PARP1 plays a protective role by preventing single-strand breaks from progressing into more severe double-strand breaks, which are more challenging for the cell to repair.

PARP Inhibition and Cancer Therapy

Due to its critical role in DNA repair, PARP1 has become a target for cancer therapy. PARP inhibitors (PARPi) prevent PARP1 from repairing DNA damage, leading to accumulation of DNA damage in cancer cells, particularly those with existing DNA repair defects, such as BRCA1/2 mutations. This strategy exploits the concept of "synthetic lethality," selectively killing cancer cells while sparing normal cells.

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In summary, PARP1 is a central component of the single-strand break repair pathway, detecting damage, coordinating the recruitment of repair proteins, and facilitating the proper resolution of DNA breaks. Its role is crucial for maintaining genome stability and is a target for therapeutic interventions, especially in cancer treatment.

Method

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, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, $F(ab')_2$ (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 980 Confocal **with NIR Detection**, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 140 of 256 total - *Panel A*

Z Stack - (3D) - *Panels A & B*

Z-Stack = 256 Slices (5.4 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 10.00 nm
Image Size (Pixels) = 601 X 708
Image Size (Scaled) = 21.21 µm X 24.99 µm
Bit Depth = 16 Bit
Image Center Position = X: 90.38 mm, Y: 46.45 mm
Stage Position = X: 90.38 mm, Y: 46.45 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - *Panels C, D, & E*

Z-Stack = 256 Slices (5.4 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 10.00 nm
Image Size (Pixels) = 294 X 252
Image Size (Scaled) = 10.48 µm X 8.89 µm
Bit Depth = 16 Bit
Image Center Position = X: 90.38 mm, Y: 46.45 mm
Stage Position = X: 90.38 mm, Y: 46.45 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

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Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS LP 640
Laser Wavelength = 639 nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82 µs
Frame Time = 6.25 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 = 643-735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 10.0 (3D, Auto)

DAPI -

Reflector - None
Contrast Method - Fluorescence
Pinhole = 4.97 AU / 214 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82 µs
Frame Time = 6.25 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 350-499

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Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 7.8 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode “SR”
Super Resolution Auto Filter Selected
Process 3D Current Image
Created

3D Image Processing - *Panel B*

3D Maximum Projection = Precise
Appearance = Threshold 3% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - *Panels C, D, & E*

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 125%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Panels C, D, and E present the same image, separating the 647 and DAPI channels to provide better visualization.

Summary - *Airyscan Super-Resolution Reveals Compartmentalized PARP1 Assemblies Following DNA Damage*

Panel A shows a two-dimensional optical section from the acquired z-stack (z-plane 140 of 256) depicting PARP1 detected with the Mouse anti-Human PARP1 monoclonal antibody (IgG1, 5F10/B7) and visualized using Identifyn™ SRM Alexa Fluor™ 647. DAPI marks nuclear DNA to provide spatial context relative to the nuclear boundary. A region of interest (white box) is indicated for higher-dimensional and higher-resolution analysis in Panels C–E.

Panel B presents a three-dimensional maximum-intensity projection of PARP1 following removal of the DAPI channel to facilitate structural interpretation. Imaging was performed using Airyscan super-resolution (~100–120 nm lateral resolution), which reveals organization within the PARP1-positive signal that appears as diffuse or non-

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informational under diffraction-limited modalities, including widefield, widefield SIM, and confocal microscopy. At this resolution, the previously confluent PARP1 signal begins to separate into discernible intranuclear features.

Panels C–E show three-dimensional volume projections in which the PARP1 (Alexa Fluor™ 647) and DAPI channels are rendered independently. This separation demonstrates that PARP1-enriched structures are not uniformly associated with DAPI-dense chromatin, indicating compartmentalized nuclear organization rather than diffuse chromatin binding.

Interpretive context and step-down integration. Based on PARP1's established biochemical role as a DNA damage sensor and catalytic mediator of poly(ADP-ribosyl)ation, we hypothesize that the Airyscan-resolved features represent PARP1-enriched assemblies associated with active PARylation, rather than direct visualization of individual poly(ADP-ribose) chain architecture. These assemblies likely reflect regions of localized PARP1 activation and polymer accumulation that exceed the resolving capacity of diffraction-limited imaging but are partially resolved at Airyscan super-resolution. Consistent structural motifs are observed across the PARP1 monoclonal step-down dataset, with further refinement and substructure delineation emerging only upon escalation to higher-resolution modalities (SIM^2 and single-molecule STORM).

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, $F(ab')_2$ (H + L) was pseudo-colored in Zen Software from red, the default color, to white, to provide visual contrast in Panel A.

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

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