

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™ 594 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000051

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000020

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.

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™ 594 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, [Actin Recombinant Rabbit Monoclonal Antibody \(JF47-01\)](#), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG F(ab')₂ (H + L) 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 Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, AxioCam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 80 Slices (7.9 µm)

Channels = 3
 Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
 Image size (Pixels) = 1199 X 962
 Image Size (Scaled) = 171.29 µm X 137.43 µm
 Bit Depth = 14 Bit
 Image Center Position = X: 52.30 µm, Y: 51.52 µm
 Stage Position = X: 52.30 µm, Y: 51.52 µm
 ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Single Plane (2D) - Panel B, Z-Plane 43 of 80, (Dimension Shown in Panel A)

Z Stack - (3D) - Panel C

Z-Stack = 89 Slices (8.8 µm)

Channels = 3
 Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm

Image size (Pixels) = 790 X 618
Image Size (Scaled) = 112.86 μm X 88.29 μm
Bit Depth = 14 Bit
Image Center Position = X: 52.30 μm , Y: 51.52 μm
Stage Position = X: 52.30 μm , Y: 51.52 μm
ROI Center Offset = X: 1.14 μm , Y: 0.00 μm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20.00%
Exposure Time = 200 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = AxioCam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.20 μm
Binning Mode = 2,2

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 100 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = AxioCam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 μm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465

Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.90 μm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.

Normalization, Phase Corrector, Fourier Filter, and Deconvolution **were NOT used.**

3D Image Processing - Panel A

3D Maximum Projection = Precise

Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, 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)

3D Image Processing - Panel C

3D Volume Projection = Precise

Appearance = Threshold 13% all channels, Ramp 50% 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)

Clipping Image Process - Panel C

Clipping planes are introduced in Panel B to highlight the PARP1 Monoclonal within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

X-Y = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 6%

Clip X = 0°

Clip Z = 0°

X-Z = Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -80%

Clip Y = 45°

Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 50%

Clip Y = -45°

Clip Z = 0°

Summary – *PARP1 Recruitment Assessed by Widefield SIM (Apotome)*

Panel A shows a three-dimensional maximum-intensity projection showing PARP1 detected with an Identifyfyn® primary antibody and visualized using an Identifyfyn® SRM Alexa Fluor™ 594 goat anti-mouse secondary antibody (red). Actin is visualized with an Identifyfyn® SRM Alexa Fluor™ 488 donkey anti-rabbit secondary antibody (green) and serves solely as a cytoplasmic exclusionary control, while DAPI marks nuclear DNA (blue).

Panel B displays an ROI corresponding to Z-plane 43 of 89.

Panel C demonstrates a three-dimensional volume reconstruction (wedge view) that illustrates orthogonal planes (X-Y, X-Z, Y-Z) of PARP1 following etoposide-induced DNA damage. Across all axes, the PARP1 signal is confined to the nuclear compartment, with no detectable cytoplasmic localization. A strong intranuclear PARP1 signal is observed; however, sub-nuclear structural features remain unresolved at this resolution. Imaging was performed using low-intensity LED excitation and short exposure times to preserve structural fidelity and avoid acquisition-driven signal amplification.

Acquired using widefield structured illumination microscopy (Apotome), this dataset demonstrates that three-dimensional SIM imaging enables visualization of PARP1 recruitment patterns that are not discernible in conventional widefield microscopy. While these aggregates do not resolve the higher-order substructures observed downstream in the Identifyfyn step-down workflow (Airyscan, SIM, SIM², and STORM), the signal provides a clear go / no-go readout confirming pharmacologically induced PARP1 engagement.

Biological and CDx context. PARP1 is a primary DNA damage sensor rapidly recruited to sites of DNA strand breaks, where it catalyzes poly(ADP-ribosyl)ation to initiate chromatin remodeling and coordinate DNA repair pathway activation. In this context, nuclear-restricted PARP1 recruitment following etoposide treatment serves as a robust CDx-relevant response marker, supporting pathway engagement confirmation, enrichment, and stratification decisions without premature structural interpretation.

Image Correction

Identifyfyn® SRM Alexa Fluor™ 594 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 Panels B and C.

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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