

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

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™ 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.

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

Single Plane (2D) - Z-plane 63 of 133 total - Panel A

Channels = 3
 Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
 Image Size (Pixels) = 5056 X x 5056
 Image Size (Scaled) = 66.72 µm X 66.72 µm
 Bit Depth = 16 Bit
 Image Center Position = X: 54.58 mm, Y: 35.81 mm
 Stage Position = X: 54.58 mm, Y: 35.81 mm
 ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B (3D Maximum projection), Panel C (2D Z-Plane 63 of 133)

Z-Stack =133 Slices (3.96 µm)

Channels = 3
 Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
 Image Size (Pixels) = 2003 X x 1794
 Image Size (Scaled) = 26.43 µm X 23.67 µm
 Bit Depth = 16 Bit
 Image Center Position = X: 54.58 mm, Y: 35.81 mm
 Stage Position = X: 54.58 mm, Y: 35.81 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Panel E

Z-Stack =133 Slices (3.96 μ m)

Channels = 3

Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm

Image Size (Pixels) = 1128 X x 681

Image Size (Scaled) = 14.88 μ m X 8.99 μ m

Bit Depth = 16 Bit

Image Center Position = X: 54.58 mm, Y: 35.81 mm

Stage Position = X: 54.58 mm, Y: 35.81 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

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 μ m

Binning Mode = 2,2

Laser Wavelength = 640 nm @3.00%

Frame Time = 1.59 s

Scan Direction = Unidirectional

Grating = 36.5 μ m grating

488 -

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 = 488

Channel Name = T2-Axiocam 820-SR

Excitation Wavelength = 488

Emission Wavelength = 525

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 = 0.81 μm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 μm grating

DAPI -

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 = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 μm
Binning Mode = 2,2
Laser Wavelength = 405 nm @5.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 μm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.4603 (647), 10.1277 (488), 8.4060 (DAPI)
Fitting = Fast Fit
Normalization = Auto

2-Dimensional View - Panels A, Notes ROI Shown in Panels B and C

Panel features Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L), Actin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (Fab')₂(H + L), and DAPI.

The image was taken from Z-stack Acquisition at plane 64 of 133.

3D Image Processing - Panel B

3D Maximum Projection = Precise

Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 77%, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

2-Dimensional View - Panels C, Notes ROI Shown in Panel D

Panel features Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L), and DAPI.

The image was taken from Z-stack Acquisition at plane 64 of 133

3D Image Processing - Panel D

3D Volume Projection = Precise

Appearance = Threshold 2%, Ramp 98%, Maximum 100% PARP1/647

Background = Black

Light = Brightness 82%, Azimuth 48°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Panel E - PARP1, STORM from the Same Data Set

Panel F - PARP1, Airyscan from the Same Data Set

Summary -

Panel A - Shows a two-dimensional optical section from the Lattice SIM z-stack (z-plane 65 of 133) depicting damage-induced PARP1 intranuclear organization.

Panel B - Presents a three-dimensional maximum-intensity projection of the region of interest defined in Panel A, revealing increased separation and continuity of PARP1-positive features compared with lower-resolution modalities.

Panel C - Shows a two-dimensional view at the same z-plane, highlighting discrete PARP1-associated structures that correspond to the features rendered in three dimensions in **Panel D**. These same structural motifs are reproducibly observed in both single-molecule STORM (Panel E) and Airyscan (Panel F) datasets.

Relative to Airyscan, Lattice SIM provides enhanced structural detail and improved three-dimensional organization, enabling partial resolution of PARP1 assemblies. While Lattice SIM does not achieve the nanoscale refinement observed with STORM, it represents a critical intermediate modality in which PARP1-associated structures begin to resolve in a

manner consistent with downstream single-molecule localization imaging. In this context, Lattice SIM crosses the structural resolution threshold required to move beyond binary detection, while still preserving a robust CDx go/no-go confirmation of PARP1 pathway engagement.

Image Corrections

NONE

Image by P. Raut

Image Analysis and Data Presentation by B.T. Bennett

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