

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 incubated for 1 hour at room temperature at a 1:250 dilution. 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](#). (Not Shown in Data Set)

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 (488 and DAPI not shown)
 Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
 Image Size (Pixels) = 2085 X x 1629
 Image Size (Scaled) = 27.51 µm X 21.50 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

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) = 955 X x 589
 Image Size (Scaled) = 12.60 µm X 7.77 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 C

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) = 206 X x 213

Image Size (Scaled) = 2.72 μ m X 2.81 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 μ m
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 μ m 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 μ m
Binning Mode = 2,2
Laser Wavelength = 405 nm @5.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 μ m grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM2
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
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).

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%, Ramp 0%, Maximum 100%

Background = Black

Light = Brightness 90%, Enabled Tone Mapping

Projection = View Angle 30°, Scale Z 10%, 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 2%, Ramp 80%, Maximum 100%

Background = Black

Light = Brightness 90%, Azimuth 80°, 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 D- PARP1, STORM from the Same Data Set**Summary - Step-down and CDx Integration across the PARP1 Dataset.**

Across the entire PARP1 imaging dataset acquired under micromolar etoposide-induced DNA damage conditions, PARP1 demonstrates a consistent, reproducible transition from a diffuse basal nuclear distribution to damage-associated nuclear recruitment and organization. At diffraction-limited resolutions (widefield, widefield SIM, and confocal), PARP1 activation is readily detected as increased nuclear signal intensity and aggregation, providing a robust binary go/no-go confirmation of DNA damage pathway engagement. Escalation to Airyscan super-resolution introduces the first level of structural discrimination, revealing compartmentalized PARP1-enriched assemblies that are not uniformly associated with DAPI-dense chromatin, consistent with localized sites of PARP1 activation and poly(ADP-ribosylation).

Further progression through Lattice SIM and SIM² within the Identifyn step-down framework yields incremental gains in lateral and axial resolution, reducing signal convolution and enabling partial resolution of PARP1-associated structures in three dimensions. These intermediate modalities reveal organizational features that are reproducible across imaging platforms and closely approximate patterns observed by single-molecule STORM, while stopping short of resolving discrete nanoscale subunits. Only STORM fully resolves the underlying heterogeneity and substructure of PARP1 assemblies formed through PAR chain synthesis and protein recruitment.

Biologically, these observations align with PARP1's established role as an early DNA damage sensor and catalytic mediator of poly(ADP-ribosylation), in which PARP1 activation at strand breaks drives the formation of large, dynamic protein-polymer assemblies that expand beyond the resolving capacity of lower-resolution imaging. Within a CDx context, the step-down dataset establishes PARP1 nuclear recruitment as a resolution-independent go/no-go response marker. At the same time, higher-resolution escalation enables stratification and mechanistic characterization without reliance on premature structural claims. Together, this framework supports PARP1 as a CDx-relevant

biomarker for DNA damage pathway activation, enrichment, and downstream resolution-dependent analysis in the context of pharmacologically induced genotoxic stress.

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 Panels B and C.

Image by P. Raut

Image Analysis and Data Presentation by B.T. Bennett

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