

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 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, but in sample)

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

Single Plane (2D) - Z-Plane 43 of 80 - Plus DNA Damage, Top

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) - Single Plane- Minus DNA Damage, Bottom

Channels = 1 (594)
Scaling (Per Pixel) = 0.143 µm X 0.143 µm
Image size (Pixels) = 726 X 800
Image Size (Scaled) = 108.86 µm X 114.29 µm
Bit Depth = 14 Bit
Image Center Position = X: 74.95 µm, Y: 46.10 µm

Stage Position = X: 74.95 μ m, Y: 46.10 μ m
ROI Center Offset = X: 1.14 μ m, Y: 0.00 μ m

594 - Identical Settings for Both Images

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 μ m
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.**

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 67.0), Y (Min 0 and Max 5267)**

This panel measures PARP1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~8K A.U. at its maximum.

Measured at Z-plane 43 of 80.

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 41.7 Max), Y (Min 28 and Max 766)**

This panel measures PARP1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~1K A.U. at its maximum.

Histogram Display - Plus DNA Damage, Top

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 64, Lower Threshold 0, Upper Threshold 16383

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 16383), Y (Min 0 and Max 2000)

The histogram was generated from the yellow-boxed region shown to quantify the overall PARP1 protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed signal increase 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 64, Lower Threshold 0, Upper Threshold 16383

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 16383), Y (Min 0 and Max 2000)

The histogram was generated from the yellow-boxed region shown to quantify the overall PARP1 protein within a defined nuclear area in the absence of DNA-damage induction. This measurement provides additional support for the observed signal increase relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusion -

In undamaged cells, PARP1 exhibits a diffuse nuclear distribution with low baseline signal and minimal aggregation, consistent with a negative (no-go) recruitment state reflecting basal chromatin association. Following induction of DNA damage, PARP1 rapidly redistributes into discrete intranuclear foci or aggregates, accompanied by an approximately fourfold increase in fluorescence intensity, meeting the predefined positive (go) threshold for damage-associated nuclear engagement. This transition reflects activation of the DNA damage response, consistent with PARP1's role as an early DNA damage sensor and catalytic mediator of poly(ADP-ribosyl)ation, and validates PARP1 recruitment as a binary, CDx-relevant assay readout for pathway engagement.

Image Corrections

None

Image by E.F. Simon

Image Analysis and Data Presentation by B.T. Bennett

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