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™ 555 Affinity Purified Goat Anti-Mouse, F(ab')$_2$ (H + L)
Cat ID# - SA 000040

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Rabbit, F(ab')$_2$ (H + L)
Cat ID# - SA 000076

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

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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™ 555 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™ 680 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 Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 1050 X 920
Image Size (Scaled) = 115.00 µm X 100.76 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

555 -

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 = 553
Emission Wavelength = 568
Effective NA = 1.4

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Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.88 µm
Binning Mode = 2,2

680 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20.00%
Exposure Time = 200 ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.09 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 = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - *Panel A*

The top-left panel features Actin visualized with Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Rabbit, IgG (Fab')2(H + L), the top-right panel shows Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized with Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, $F(ab')_2$ (H + L), the bottom-left panel features DAPI nuclear staining. The bottom-right panel features the merged image of all channels.

Colocalization View - *Panels B and C*

Horizontal Axis - AF555, Threshold 320, Range Auto
Vertical Axis - DAPI, Threshold 210, Range Auto
Image Measurement = Entire Field

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Colocalization analysis of PAPR1 (X-axis) and DAPI (Y-axis) was performed across the entire image acquisition. PARP1 shows strong colocalization with DAPI, consistent with localization within the nuclear compartment.

Zeiss Zen Acquisition Settings - *Panel D*

Shown are the acquisition settings across all three channels, including LED intensity and exposure times, along with all other relevant parameters, provided for methodological transparency.

Summary

Panel A - Presents a split-view of the full acquisition field, including actin detected with an Alexa Fluor™ 680 secondary antibody used solely as a cytoplasmic exclusionary control. This demonstrates that the PARP1 signal is not cytoplasmic and is instead confined to the nuclear compartment. Actin was included exclusively to delineate cytoplasmic boundaries and was not used as a biological marker in the analysis.

Panels B and C - Present orthogonal intensity profiles of PARP1 (horizontal axis) and DAPI (vertical axis), confirming exclusive nuclear localization of PARP1 following etoposide-induced DNA damage. No measurable signal is detected within the cytoplasm. Strong nucleolar-associated PARP1 aggregates are observed; however, at this stage of the step-down workflow, these structures remain spatially unresolved and are not structurally interpreted, serving instead as confirmation of compartment-restricted recruitment.

Panel D - Provides acquisition transparency, documenting low LED excitation for PARP1/Alexa Fluor™ 680 with a 200 ms exposure time. These parameters demonstrate that the observed signal is not artificially driven by acquisition settings but instead reflects a robust and specific biological response mediated by a highly specific primary antibody following pharmacologically induced DNA damage.

Biological and CDx context - PARP1 is a central DNA damage sensor rapidly recruited to sites of single- and double-strand breaks, where it catalyzes poly(ADP-ribosyl)ation to coordinate chromatin remodeling and DNA repair pathway engagement. In this context, nuclear-restricted PARP1 recruitment and aggregation following etoposide treatment provides a clear go / no-go confirmation of DNA damage pathway activation within the Identifyn step-down framework. While higher-resolution modalities are required to resolve sub-nucleolar architecture, the current data establish PARP1 as a robust companion-diagnostic (CDx)–relevant response marker, suitable for pathway engagement, enrichment, and stratification decisions without premature structural over-interpretation.

Image Correction

None

Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

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