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 LSM 980 Confocal **with NIR Detection**, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Single Plane (2D) - Z-pane 58 of 155 - *Panel A*

Channels = 3
Scaling (Per Pixel) = 0.030 µm X 0.030 µm X 0.030 µm
Image size (Pixels) = 1276 X 1279
Image Size (Scaled) = 33.77 µm X 33.77 µm
Bit Depth = 16 Bit
Image Center Position = X: 91.35 µm, Y: 46.46 µm
Stage Position = X: 91.35 µm, Y: 46.46 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - *Panels B and C*

Z-Stack = 144 Slices (4.29 µm)

Channels = 3
Scaling (Per Pixel) = 0.030 µm X 0.030 µm X 0.030 µm
Image size (Pixels) = 1276 X 1279
Image Size (Scaled) = 33.77 µm X 33.77 µm
Bit Depth = 16 Bit
Image Center Position = X: 91.35 µm, Y: 46.46 µm

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Stage Position = X: 91.35 µm, Y: 46.46 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 55 µm
LSM MBS = MBS 488/561/639, 405/730
LSM SBS = Mirror
Laser Wavelength = **561 nm @0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.38
Pixel Time = 0.26µs
Frame Time = 2.10s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 550 - 600
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.5 (3D, Auto)

680 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 68µm
LMS MBS = MBS 488/561/639 MBS 405/730
LMS SBS = SBS SP 615
Laser Wavelength = **639nm @0.4%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.38
Pixel Time = 0.26 µs
Frame Time = 2.10s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4

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Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 667 - 750
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.8 (3D, Auto)

DAPI -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 31µm
LMS MBS = MBS 488/561/639 MBS 405/730
LMS SBS = Mirror
Laser Wavelength = **405nm @0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.38
Pixel Time = 0.26 µs
Frame Time = 2.10s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410 - 469
Imaging Device = GaAsP-PMT3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.7 (3D, Auto)

2-Dimensional View - *Panel A*

Panel features Actin visualized with Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Rabbit, IgG (Fab')2(H + L), 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), and DAPI.

The image was taken from Z-stack Acquisition at plane 58 of 144.

3D Image Processing - *Panel B*

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3D Maximum Projection = Precise
Appearance = Threshold 2% 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 1% (555), Ramp 98% (555), Maximum 100%
Background = Black
Light = Brightness 125%, 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)

Summary -

Two-dimensional projection showing PARP1 detected with a monoclonal primary antibody and visualized using Identifyn® SRM Alexa Fluor™ 555 goat anti-mouse secondary antibody (orange), actin detected with a rabbit recombinant antibody and visualized using Identifyn® SRM Alexa Fluor™ 680 donkey anti-rabbit secondary antibody (green), and DAPI nuclear staining (blue). Actin serves exclusively as a cytoplasmic exclusionary control.

Three-dimensional maximum-intensity and volume projections of PARP1 and DAPI reveal the intranuclear distribution of signals following etoposide-induced DNA damage.

When imaged by conventional confocal microscopy, PARP1 damage-induced aggregates remain unresolved due to diffraction-limited signal convolution. Airyscan imaging, which provides moderate super-resolution (~120–140 nm lateral resolution), improves signal-to-noise and enables partial feature separation; however, finer structural organization remains unresolved. More complete structural delineation emerges only after escalation to higher-resolution super-resolution modalities, including SIM^2 and, most clearly, single-molecule STORM.

This resolution dependence is biologically expected, as PARP1 rapidly synthesizes extended poly(ADP-ribose) chains upon activation, generating large, dynamic protein–polymer assemblies whose internal organization exceeds the resolving capacity of confocal and Airyscan imaging.

In this framework, confocal and Airyscan provide reliable go / no-go confirmation of PARP1 pathway activation, while SIM^2 and STORM are required to interrogate the underlying nanoscale organization of PARP1 assemblies relevant to CDx stratification.

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

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