

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M)***Cat ID# - PA-RR 000003*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)***Cat ID# - SA 000041***Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000075*

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with [Thermo Fisher Fish Serum Blocker cat#37527X3](#). The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. 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:

Z Stack - (3D)**Z-Stack = 146 Slices (4.35µm)**

Channels = 3

Scaling (Per Pixel) = 0.030µm X 0.030µm X 0.030µm

Image Size (Pixels) = 1276 X 1276
Image Size (Scaled) = 37.77 μ m X 37.77 μ m
Bit Depth = 16 Bit
Image Center Position = X: 88.97mm, Y: 49.88mm
Stage Position = X: 88.97mm, Y: 49.88mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 55 μ m
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 561nm @0.1%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.5
Rotation = 0°
Sampling = 2.38
Pixel Time = 0.26 μ s
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-Pmt 3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.4 (3D, Auto)

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 68 μ m
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 639nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.5
Rotation = 0°
Sampling = 2.38
Pixel Time = 0.26 μ s
Frame Time = 2.10s

LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 667 - 750
Imaging Device = GaAsP-Pmt 3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.5 (3D, Auto)

DAPI - *(Not Shown, in mounting medium)*

Reflector - None
Contrast Method - Fluorescence
Pinhole = 0.74 AU / 31µm
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Scan Zoom = 3.5
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 = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410 - 469
Imaging Device = GaAsP-Pmt 3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.7 (3D, Auto)

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels.
Background = Black

Light = Brightness100%, Tone Mapping NOT Enabled

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.

X-Y = Active was selected, textured opaque was chosen to remove a horizontal portion of the upper Z-Stack of the cell from the image, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected. The cut plane was used to show the specificity, localization, and brightness of the Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG (BLR018M) combined with Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Rabbit, IgG (H + L) Cat ID# - SA 000035.

Position of Clip = -20%

Clip X = 0°

Clip Y = 0°

Summary -

Panel A – This panel presents a 2-dimensional representation of image acquisition at Z-plane 74 of 146, illustrating the localization of PARP1 within the nuclear compartment.

Panel B – Here, we provide a 3-dimensional maximum projection of the complete image acquisition, consisting of 146 individual Z-planes. This panel features a cut plane at Z-plane 74, showcasing both the 3D modeling and localization in this perspective.

Panel C – In this panel, we display the settings used on the Zeiss LSM980 to acquire this image. We note that the Identifyn® PARP1 Rabbit Recombinant Monoclonal Antibody, combined with Identifyn® SRM Alexa Fluor™ 555 Goat anti-Rabbit, F(ab')₂ (H+L), required only 0.1% excitation power from the 561 nm laser to produce the image. Additionally, the Identifyn® SRM Alexa Fluor™ 680 Donkey anti-Mouse, F(ab')₂ also contributed, utilizing 0.3% of the 639 nm laser input. We highlight the sampling, pixel frame times, and scan speed used for this image, along with a low averaging of 4. Furthermore, we present the LSMP settings (LSM Plus) to demonstrate the image quality.

Conclusion – The Identifyn® PARP1 Rabbit Recombinant antibody exhibits excellent binding to PARP1 in the nuclear compartment and delivers high brightness when paired with Identifyn® secondary antibodies, enabling clear visualization of localization. Employing LSM Plus, it can be argued that the structural details of the aggregates begin to provide additional information. However, we make no claims regarding biological implications; it does, however, provide a query for further study.

Image Corrections

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse, IgG (H + L), Cat ID# - SA 000070 was psuedo colored in ZEN software from the default color, dark red, to white to provide better visual contrast.

Image by J.K. Bennett

Analysis and Presentation by B.T. Bennett

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