

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™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000019***Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)***Cat ID# - SA 000063*

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® 647 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® 488 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 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)**Z - Stack = 123 Slices (12.2µm)**

Channels = 3

Scaling (Per Pixel) = 0.143µm X 0.143µm x 100µm

Image Size (Pixels) = 751 X 885
Image Size (Scaled) = 107.29µm X 126.43µm
Bit Depth = 14 Bit
Image Center Position = X: 62.38mm, Y: 50.80mm
Stage Position = X: 62.38mm, Y: 50.80mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.0%
Exposure Time = 150ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = AxioCam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00µm
Binning Mode = 2,2

647 -

Reflector = 350 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 665
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @6.0%
Exposure Time = 80ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = AxioCam 807
Camera Adapter = 1X
Depth of Focus = 1.30µm
Binning Mode = 2,2

DAPI - *(Not Shown but stained in mounting media)*

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 6.0%%
Exposure Time = 18ms
Excitation Wavelength = 353
Emission Wavelength = 465

Effective NA = 1.25
Imaging Device = AxioCam 807
Camera Adapter = 1X
Depth of Focus = 0.90µm
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.

3D Image Processing - Panels A and C

3D Maximum Projection = Precise
Appearance = Threshold 13% for all channels, Ramp 0% for all channels, and Maximum 100% for all channels.
Background = Black
Light = Brightness 100%, **Tone Mapping was 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)

Profile View Display - Panel B

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 18.6), Y (Min 2 and Max 4340)

Summary

Panel A – Displays a 2D view of Z-Plane 60 out of 123, with the main image showing a 3D Maximum Projection View of the entire acquisition. A circle indicates an ROI and is used in Panels B and C.

Panel B – Features a line profile across the nucleus selected in Panel A, indicating that PARP1 staining is localized within the nuclear compartment, with intensity measured on the Y-axis.

Panel C – Provides a 3D maximum projection of the selected region of interest, as shown in the line profile in Panel B.

Panel D – Demonstrates the use of Identifyn® Rabbit Recombinant PARP1 and Identifyn® SRM Alexa Fluor™ 647 at a 6.0% light intensity and 80 ms exposure, yielding robust staining and signal detection.

Image Corrections

None

Image by E.F. Simon

Analysis and Presentation by B.T. Bennett

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