

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:

Single Plane (2D) - Panel A

Channels = 3

Scaling (Per Pixel) = 0.143µm X 0.143µm

Image Size (Pixels) = 971 X 773

Image Size (Scaled) = 138.71µm X 110.43µm

Bit Depth = 14 Bit

Image Center Position = X: 52.81mm, Y: 47.64mm
Stage Position = X: 52.81mm, Y: 47.64mm
ROI Center Offset = X: 1.14μm, Y: 0.00μm

Single Plane (2D) - Panel B

Channels = 3
Scaling (Per Pixel) = 0.143μm X 0.143μm
Image Size (Pixels) = 930 X 813
Image Size (Scaled) = 132.86μm X 116.14μm
Bit Depth = 14 Bit
Image Center Position = X: 60.90mm, Y: 54.88mm
Stage Position = X: 60.90mm, Y: 54.88mm
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 -

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.

Summary

Panel A – Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M) in the absence of Etoposide.

Panel B – Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M) treated with Etoposide.

Panel C – Western Blot, minus/plus etoposide-induced DNA damage.

Note - The settings for both images in Panels A and B were the same, using a Zeiss Axio Observer.Z1/7 Apotome SIM with an EC Plan-Neofluar 63X/1.25 oil M27 objective, an Axiocam 807, and an X-Cite Xylis Lamp.

As observed in the Western Blot, the appearance of PARP1 in both the undamaged and damaged lanes increases in response to the presence of Etoposide. Similar results are seen in the control images.

Image Corrections

None

Image by E.F. Simon

Image Analysis and Data Presentation by B.T. Bennett

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