

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, IgG (H + L)***Cat ID# - SA 000035***Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000070*

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

Single Plane (2D) - Split View Panel A

Channels = 3

Scaling (Per Pixel) = 0.110µm X 0.110µm

Image size (Pixels) = 1216 X 1028

Image Size (Scaled) = 133.18µm X 112.59µm

Bit Depth = 14 Bit

Image Center Position = X: 0.00 μ m, Y: 0.00 μ m
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

680 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 400ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 1.09 μ m
Binning Mode = 2,2

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 @12%
Exposure Time = 100ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 0.88 μ m
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 @15%
Exposure Time = 20ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 0.72 μ m

Binning Mode = 2,2

Post Processing

None

Summary

This data point presents a widefield image of the Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal IgG. The imaging methods highlight that the PARP1 antibody was visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Rabbit IgG (H + L), Cat ID# SA 000035. This was followed by primary staining against alpha-tubulin, using Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse IgG (H + L), Cat ID# SA 000070. The nucleus was stained with DAPI following etoposide-induced DNA damage.

The PARP1 anti-Human Rabbit Recombinant antibody was produced against synthetic peptides corresponding to variable regions within human PARP1. The Western Blot data demonstrate that the antibody effectively recognizes the DNA damage response of the population we chose to image.

The image is presented in a split-view format: Panel A shows PARP1 staining in the upper left corner, alpha-tubulin staining in the upper right panel, DAPI staining in the lower left, and the merged image of all channels in the lower right. The split view is crucial for accurately visualizing localization within the nuclear compartment. In widefield applications, accurate localization can aid in studies of both population and individual nuclei.

Panel B features a clipped image directly from the Zeiss Zen software, providing transparency regarding the image acquisition. This ensures clarity in demonstrating the acquisition settings and highlights the robust signal produced by the combinatorial use of the Identifyn® PARP1 primary antibody and the Identifyn® SRM Alexa Fluor™ secondary conjugates, which exhibit excellent brightness with minimal LED input and exposure times.

Image Correction

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 E.F. Simon

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