Identifyn Standards for Optical Microscopy Methods™
Identifyn® Primary Antibody
Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')$_2$ (H + L)
Cat ID# - SA 000017

p21, CDKN1A (cyclin-dependent kinase inhibitor 1A), is a regulatory protein with multiple roles in cell cycle control, DNA damage response, and repair. It is a well-known target of the tumor suppressor protein p53, and its regulation is crucial for cellular responses to DNA damage. The functions of p21 in the context of DNA repair:

Cell Cycle Arrest: p21 is a crucial mediator of cell cycle arrest, particularly in response to DNA damage. It inhibits cyclin-dependent kinases (CDKs), specifically CDK2, CDK4, and CDK6, halting cell cycle progression in the G1 phase. This arrest allows cells time to repair damaged DNA before cell division, reducing the risk of transmitting DNA errors.

Role in DNA Repair: p21, primarily known for its role in cell cycle regulation, can also influence DNA repair mechanisms:

Coordination of Repair Pathways: Inducing cell cycle arrest, p21 provides a stable environment for DNA repair processes. This action helps ensure that repair mechanisms, such as homologous recombination (HR) and nucleotide excision repair (NER), have the time and resources needed to repair DNA damage effectively.

Interacting with DNA Repair Proteins: p21 can interact with various DNA repair proteins, affecting their stability and activity. For example, p21 can bind to proliferating cell nuclear antigen (PCNA), a key component in DNA replication and repair. This interaction can modulate PCNA's activity and help regulate the balance between DNA repair and replication.

Protection from Apoptosis: In some contexts, p21 can protect cells from undergoing apoptosis, providing additional time for DNA repair. This role is complex and context-dependent, as it can also contribute to cellular resistance to specific cancer therapies by prolonging the survival of damaged cells.

Role in Genomic Stability: By facilitating cell cycle arrest and enabling effective repair pathways, p21 helps maintain genomic stability. This role is crucial for preventing mutations and chromosomal aberrations leading to cancer and other genetic diseases.

In summary, p21 plays a multifaceted role in DNA repair by regulating cell cycle progression, coordinating with DNA repair proteins, and providing a stable environment for repair processes. Its ability to interact with components of the cell cycle and the DNA repair machinery makes it a crucial mediator of the cellular response to DNA damage, contributing to genomic integrity and stability.

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Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 24 hours, followed by fixation in -20°C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human p21 Recombinant Monoclonal, IgG (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, $F(ab')_2$ (H + L) was incubated for 1 hour at room temperature 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) - *Panel A*
Z-Stack = 81 Slices (8.0 µm)

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 870 X 826
Image Size (Scaled) = 124.29 µm X 118.00 µm
Bit Depth = 14 Bit
Image Center Position = X: 45.68 mm, Y: 50.45 mm
Stage Position = X: 45.68 mm, Y: 50.45 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - *Panel B, ROI*
Z-Stack = 81 Slices (8.0 µm)

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 284 X 214
Image Size (Scaled) = 35.43 µm X 30.57 µm
Bit Depth = 14 Bit
Image Center Position = X: 45.68 mm, Y: 50.45 mm
Stage Position = X: 45.68 mm, Y: 50.45 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

647 -
Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715

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Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @ 30.00%
Exposure Time = 300 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.30 µm
Binning Mode = 2,2

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 @ 25.00%
Exposure Time = 250 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
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**

3D Image Processing - *Panel A*

3D Maximum Projection = Precise
Appearance = Threshold 13% 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 B*

3D Maximum Projection = Precise
Appearance = Threshold 13% 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)

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Clipping Image Process - *Panel B*

Clipping planes are introduced in Panel B to highlight the Rabbit anti-Human p21 Recombinant Monoclonal, IgG (BLR022N) and the Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Within the nuclear compartment, by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.
X-Y = Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip = -31%
Clip X = 0°
Clip Z = 0°
X-Z = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip = -13%
Clip Y = 30°
Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a violet outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip = -11%
Clip Y = -45°
Clip Z = 0°

Summary

Panel B presents a wedge-cut, volume-rendered view from a top-down perspective with slight posterior rotation, exposing the region of wedge removal and enabling visualization of signal distribution throughout the z-stack. This orientation provides complementary geometric context and supports spatial colocalization of p21 and PARP1 following etoposide-induced DNA damage. Based on the confined, centrally localized signal morphology—consistent with expected nuclear distribution under these conditions—the observed colocalization likely occurs within the nuclear compartment. The volumetric reconstruction and orthogonal perspective further exclude peripheral or out-of-plane signal contributions, confirming that the observed overlap is not due to projection or boundary artifacts.

Image Correction

NONE

Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

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