

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 that lead 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 the repair process. 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.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 μ M 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 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) - 2D at Z-Plane 67 of 141, Panel A

Z-Stack = 141 Slices (4.2 μ m)

Channels = 2
 Scaling (Per Pixel) = 35.0 nm X 35.0 nm X 30.0 nm
 Image size (Pixels) = 720 X 996
 Image Size (Scaled) = 25.41 μ m X 35.14 μ m
 Bit Depth = 16 Bit
 Image Center Position = X: 82.17 mm, Y: 53.61 mm
 Stage Position = X: 82.17 mm, Y: 53.61 mm
 ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - 3D Volume Projection, Panel B

Z-Stack = 141 Slices (4.2 μ m)

Channels = 2
 Scaling (Per Pixel) = 35.0 nm X 35.0 nm X 30.0 nm
 Image size (Pixels) = 333 X 346
 Image Size (Scaled) = 11.75 μ m X 12.21 μ m
 Bit Depth = 16 Bit
 Image Center Position = X: 82.17 mm, Y: 53.61 mm
 Stage Position = X: 82.17 mm, Y: 53.61 mm
 ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

647

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328 μ m
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = **639nm @ 0.9%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 μ s
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 10.0 (3D, Auto)

488

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250 μ m
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **488nm @ 0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 μ s
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548

Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 9.3 (3D, Auto)

DAPI - *Not Shown in Data Analysis, but Collected in Image Acquisition*

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 235 μ m
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **405nm @ 0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 μ s
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 420 - 480, 495 - 499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.6 (3D, Auto)

2-Dimensional View - *Panel A*

Shows Rabbit anti-Human p21 Recombinant Monoclonal, IgG (BLR022N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L). A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - *Panel B*

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black

Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Panel C shows STORM imaging from the same product dataset to demonstrate the resolution limitations of Airyscan, which are improved relative to conventional confocal imaging, in determining localization and structural details.

Summary

Panel A shows a single optical section (Z-plane 67/141) depicting p21 and PARP1 distribution following etoposide-induced DNA damage. Panel B provides a 3D volume reconstruction of the ROI from Panel A, illustrating the spatial proximity of p21 and PARP1 within the imaged volume. While apparent overlap is observed at the diffraction-limited scale, these signals are not expected to exhibit direct molecular colocalization, as p21 functions in downstream cell-cycle regulation, whereas PARP1 acts as an early DNA-damage sensor.

Conventional and Airyscan imaging remain resolution-limited, potentially conflating closely apposed but distinct structures. Panel C shows STORM imaging of p21 alone, revealing refined structural details and sub-diffraction organization not resolved in lower-resolution modalities. These data support that the apparent overlap observed in Panels A and B likely reflects spatial proximity rather than true molecular colocalization.

Image Correction

NONE

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.