

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N)***Cat ID# - PA-RR 000009*Identifyn® Secondary Antibody**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000060*

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.

Methods**Plus Damage -**

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via 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 and mounted using [ProLong™ Diamond Antifade Mountant with DAPI \(cat# P36962\)](#).

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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 100 of 201, Panel A, from Confocal Data Set

Z-Stack = 201 Slices (6.0 µm)

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 573 X 656
Image Size (Scaled) = 33.70 µm X 35.58 µm
Bit Depth = 16 Bit
Image Center Position = X: 81.04 mm, Y: 53.39 mm
Stage Position = X: 81.04 mm, Y: 53.39 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Control

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm
Image size (Pixels) = 1006 X 732
Image Size (Scaled) = 59.16 µm X 43.05 µm
Bit Depth = 16 Bit
Image Center Position = X: 72.53 mm, Y: 51.01 mm
Stage Position = X: 72.53 mm, Y: 51.01 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 - Plus Damage, from Confocal Data Set

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 64 µm

LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = **639nm @ 0.10%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 µs
Frame Time = 4.97 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.6 (3D, Auto)

647 - *Minus Damage - noting the same laser input power and conditions*

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 64 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = **639nm @ 0.10%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.25 µs
Frame Time = 10.24 s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V

Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.3 (3D, Auto)

Profile View Display - *Plus DNA Damage, Top*

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - **X (Min 0.0 and Max 32.60), Y (Min 0.0 and Max 6129)**

This panel measures p21 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~6K A.U. at its maximum.

Measured at the Z-plane 100 of 201.

Profile View Display - *Minus DNA Damage, Bottom*

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - **X (Min 0.0 and Max 53.80), Y (Min 0.0 and Max 366)**

This panel measures p21 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~300 A.U. at its maximum.

Histogram Display - *Plus DNA Damage, Top*

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 5000)

Arithmetic Mean intensity = 1384.301

The histogram was generated from the yellow-boxed region shown to quantify the overall p21 response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - *Minus DNA Damage, Bottom*

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 100000)

Arithmetic Mean intensity = 45.598

The histogram was generated from the yellow-boxed region shown to quantify the overall p21 protein signal within a defined nuclear area in the absence of DNA-damage induction. This measurement provides a baseline comparison for the increased signal observed following DNA-damage induction, complementing the line-profile analysis presented alongside it.

Control Conclusion –

In undamaged cells, p21 exhibits low-intensity, diffuse distribution, consistent with basal expression and limited activation of cell cycle checkpoint pathways. Following etoposide-induced DNA damage, p21 shows a marked increase in mean intensity. It reorganizes into discrete nuclear foci, with an aggregate signal elevated by ~30.4-fold. This substantial increase is consistent with robust activation of the DNA damage response, reflecting p53-mediated transcriptional upregulation of p21 and subsequent engagement of cell cycle arrest mechanisms.

The pronounced increase in signal intensity and focal organization aligns with established kinetics of p21 accumulation following genotoxic stress, supporting its role as a downstream effector of DNA damage signaling rather than an initial damage sensor.

Image Corrections

None

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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