BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

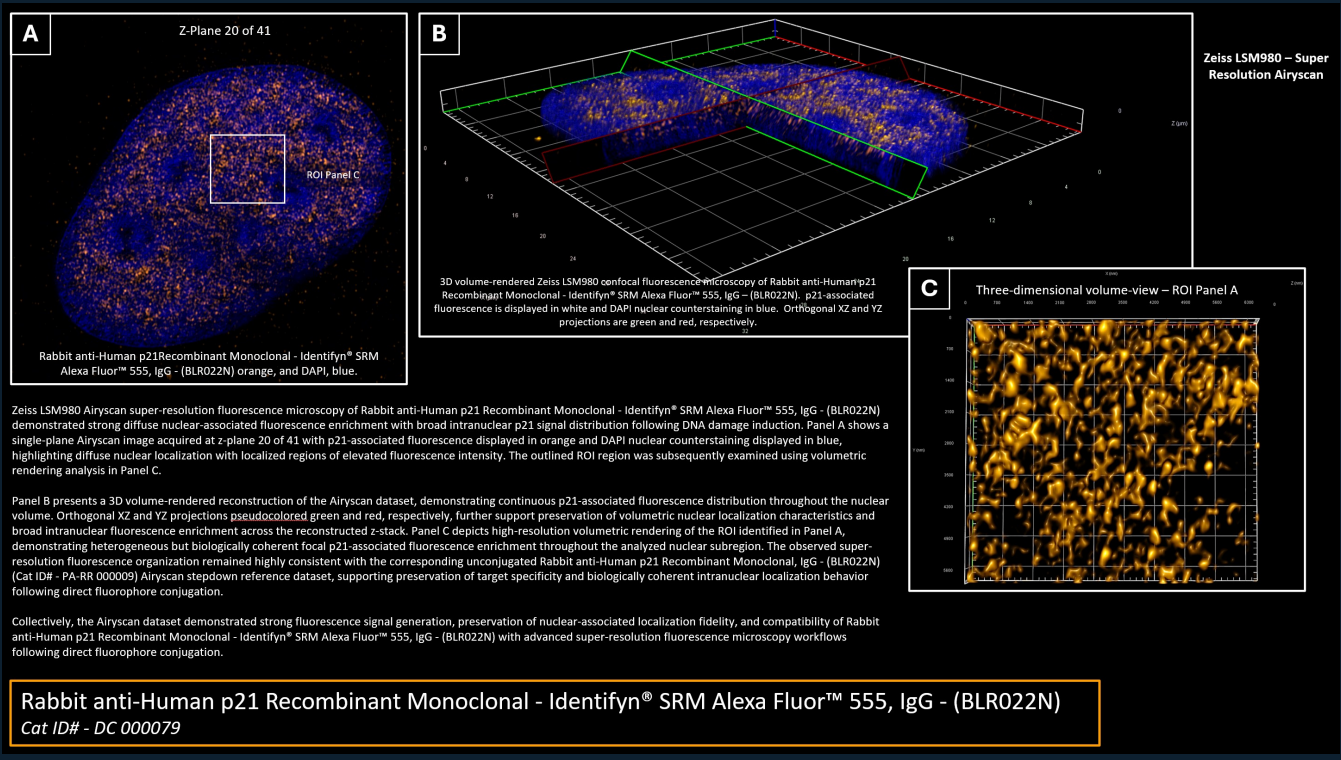
P21

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555

WESTERN BLOT -> WIDEFIELD -> CONFOCAL -> AIRYSCAN

4	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Zeiss LSM980 Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) demonstrated strong diffuse nuclear-associated fluorescence enrichment with broad intranuclear p21 signal distribution following DNA damage induction. Panel A shows a single-plane Airyscan image acquired at z-plane 20 of 41 with p21-associated fluorescence displayed in orange and DAPI nuclear counterstaining displayed in blue, highlighting diffuse nuclear localization with localized regions of elevated fluorescence intensity. The outlined ROI region was subsequently examined using volumetric rendering analysis in Panel C.

Panel B presents a 3D volume-rendered reconstruction of the Airyscan dataset, demonstrating continuous p21-associated fluorescence distribution throughout the nuclear volume. Orthogonal XZ and YZ projections pseudocolored green and red, respectively, further support preservation of volumetric nuclear localization characteristics and broad intranuclear fluorescence enrichment across the reconstructed z-stack. Panel C depicts high-resolution volumetric rendering of the ROI identified in Panel A, demonstrating heterogeneous but biologically coherent focal p21-associated fluorescence enrichment throughout the analyzed nuclear subregion. The observed super-resolution fluorescence organization remained highly consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) (Cat ID# - PA-RR 000009) Airyscan stepdown reference dataset, supporting preservation of target specificity and biologically coherent intranuclear localization behavior following direct fluorophore conjugation.

Collectively, the Airyscan dataset demonstrated strong fluorescence signal generation, preservation of nuclear-associated localization fidelity, and compatibility of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) with advanced super-resolution fluorescence microscopy workflows following direct fluorophore conjugation.

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N)
Cat ID# - DC 000079

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / AIRYSCAN

DC-000079 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

Identifyn performed the experimental and imaging work documented below. BENNETT converts that operating history into a governed, source-bounded scientific record.

- Source-documented sample preparation and antibody/reagent workflow
- Western blot imaging
- Widefield fluorescence microscopy
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for P21, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Confocal -> Airyscan

BENNETT ASSESSMENT

The preserved DC-000079 record contains Western blot; Widefield; Confocal; Airyscan. Missing modalities are not represented or inferred.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

SOURCE STATE	SOURCE HASH VERIFIED / EVENT RECORDED
BENNETT EVIDENCE REVIEW	COMPLETED / SOURCE-BOUNDED
NATIVE IMAGE REPROCESSING	NOT PERFORMED
LITERATURE SOURCES	INDEPENDENTLY VERIFIED
SCIENTIST REVIEW	PENDING
PUBLICATION	NOT AUTHORIZED

OVERALL CALL / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000079 record contains Western blot; Widefield; Confocal; Airyscan. Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 4 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

The record supports retrospective, source-bounded microscopy review. Target identity, specificity, treatment effect, binding, interaction and mechanism are not inferred from presentation images alone.

CONTROL ASSESSMENT

No separate control record is preserved; none is inferred from method or template language.

CLAIM CEILING

This draft supports historical capability documentation, source registration, modality ordering, settings review and bounded interpretation. It does not independently validate antibody specificity, quantify a population response, establish binding, interaction, causation or mechanism, or authorize publication.

PROCESSING DISCLOSURE

Retrospective BENNETT architectural evaluation of preserved Identifyn source evidence. BENNETT did not perform native-file reprocessing, new deterministic measurement or the historical image post-processing represented in this record.

REAGENTS AND ACQUIRED CHANNELS

Preparation reagents are reported separately from channels actually documented in the acquisition settings. Fluorophores mentioned in staining are not automatically treated as acquired channels.

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	532; 555	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555	2; 45 Texas Red; 49 DAPI; 540 - 580; 335 - 383; 593 - 668; 420 - 470; 553
Confocal	405/DAPI; 488; 555	2; None; 561 nm @ 2.00%; 405 nm @ 0.3%; 553; 353; 568; 465
Airyscan	405/DAPI; 488; 555	2; None; 561 nm @ 2.00%; 405 nm @ 0.3%; 553; 353; 568; 465

MODALITY ASSESSMENT / PART 1 OF 4

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Azure Sapphire FL. Objective: not explicitly stated. Platform: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.. Structured source metadata values: 11.

VISIBLE OBSERVATION

A preserved presentation image is available for Western blot. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 532; 555.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Band position and displayed intensity do not independently establish analytical specificity, target identity, linearity, or treatment effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Western blot evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 2 OF 4

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 793 X 691; Image Size (Pixels): 793 X 691; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 250 ms; 25 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 25.00%; X-Cite Xylis Lamp Intensity @ 10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 40.

VISIBLE OBSERVATION

A preserved presentation image is available for Widefield. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 555.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

A presentation image supports gross localization and source-bounded co-occurrence, not direct binding, molecular colocalization, or native quantitative measurement.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Widefield evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 3 OF 4

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: 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:. Acquisition: Z-Stack: 51 Slices (1.50 µm); Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 754 X 734; Image Size (Pixels): 754 X 734; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.29 µs; Frame Time: 4.97 s. Illumination: Laser Wavelength: 561 nm @ 2.00%; 405 nm @ 0.3%. Optical/scan: Pinhole: 1.00 AU / 55 µm; 1.00 AU / 44 µm; Scan Zoom: 1.7; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.9 (3D, Auto); Filter: 6.8 (3D, Auto). Structured source metadata values: 45.

VISIBLE OBSERVATION

A preserved presentation image is available for Confocal. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 555.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Optical sectioning and source-bounded 3D presentation do not establish binding, mechanism, or population-level effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Confocal evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 4 OF 4

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: 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:. Acquisition: Z-Stack: 41 Slices (1.20 µm); Channels: 2; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 1005 X 940; 186 X 168; Image Size (Pixels): 1005 X 940; 186 X 168; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.10 µs; Frame Time: 18.77 s. Illumination: Laser Wavelength: 561 nm @ 2.00%; 405 nm @ 0.3%. Optical/scan: Pinhole: 5.00 AU / 309 µm; 5.00 AU / 219 µm; Scan Zoom: 2.0; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 35°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: SuperResolution: 10.0 (3D, Auto); SuperResolution: 9.3 (3D, Auto). Structured source metadata values: 60.

VISIBLE OBSERVATION

A preserved presentation image is available for Airyscan. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 555.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Displayed feature separation remains reconstruction dependent; no unsupported numerical resolution or molecular architecture claim is permitted.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Airyscan evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

CROSS-MODALITY SYNTHESIS

The preserved DC-000079 record contains Western blot; Widefield; Confocal; Airyscan. Missing modalities are not represented or inferred.

Different acquisitions are not treated as a quantitative intensity ladder. Any continuity statement remains qualitative and source bounded.

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

BENNETT uses independently verified primary scientific sources to test whether the interpretation is scientifically consistent and properly bounded. Literature correlation does not retroactively validate the Identifyn dataset or replace scientist review.

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.

- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

1. Uhlen M, et al. A proposal for validation of antibodies. Nat Methods. 2016;13:823-827. doi:10.1038/nmeth.3995.
2. Gustafsson MGL. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc. 2000;198:82-87. doi:10.1046/j.1365-2818.2000.00710.x.
3. Sage D, et al. Quantitative evaluation of software packages for single-molecule localization microscopy. Nat Methods. 2015;12:717-724. doi:10.1038/nmeth.3442.

RELEASE BOUNDARY

This assessment remains a draft pending scientist review. No publication is authorized. Any future native-file analysis must enter BENNETT as a new evidence snapshot with routed engine authority, native-data QA, methods, limitations, and an independently reviewed claim ceiling.

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

Historical Identifyn source material was subjected to BENNETT retrospective QA. Original source state and correction history remain preserved in provenance.

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 µm X 0.03530 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1005 X 940; Image Size (Scaled)=35.47 µm X 33.18 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

BENNETT has completed the bounded retrospective assessment.
The following pages switch ownership to the preserved Identifyn record.

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked Stepdown order. Scientific wording is preserved; missing modalities are not invented. No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

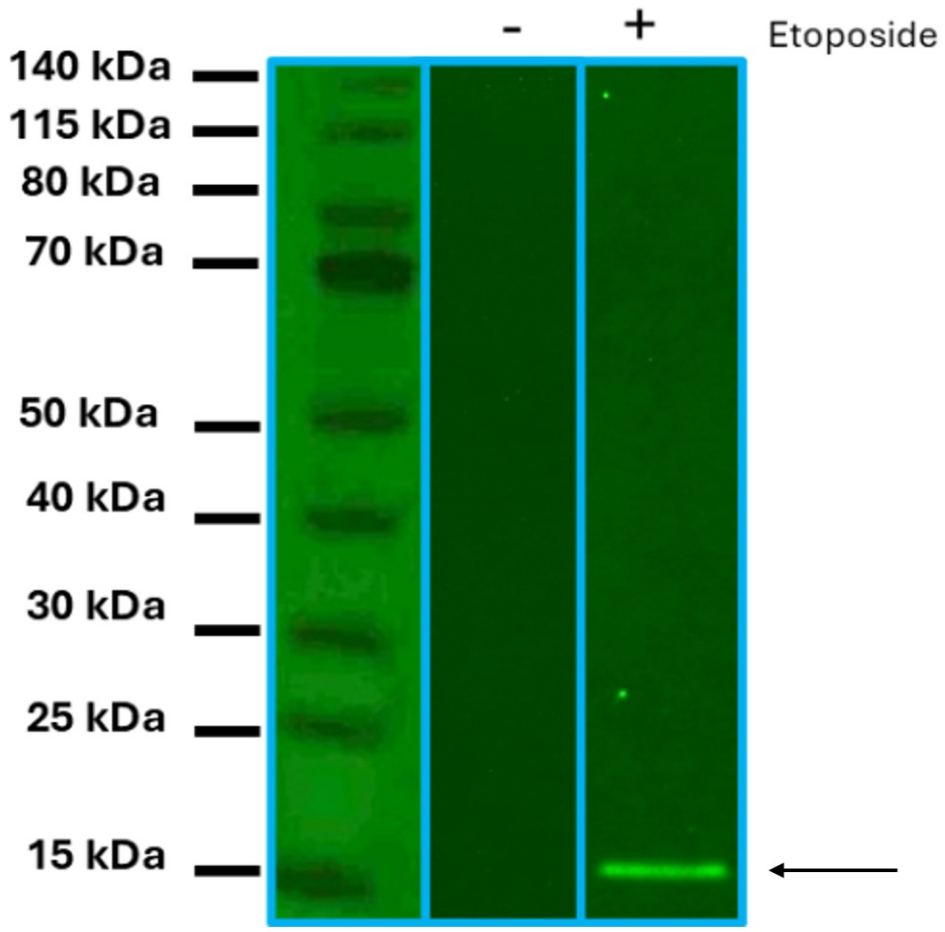
This dossier preserves the complete available evidence progression for DC-000079. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED

INTERPRETIVE BOUNDARY

Ordering evidence by resolution tier does not make the modalities interchangeable. Each stage retains its acquisition environment and scientific limitations. This pilot organizes the record; it does not synthesize new biological claims.

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	532; 555
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Detection mode	Fluorescence
Laser	532 nm
Emission Filter	572 ± 28nm
Detector	PMT
Intensity	L2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N)
Cat ID# - DC 000079

Expected MW: 21 kDa

HeLa cells were treated with 2 µM etoposide for 12 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ 4 to 12% Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank. ThermoFisher™ PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour, then washed 5 times with PBS. The blocked membrane was incubated with Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) for 20 hours, followed by five washes in 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.

Scan Settings

Detection mode = Fluorescence
Laser = 532 nm
Emission Filter = 572 ± 28nm
Detector = PMT
Intensity = L2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

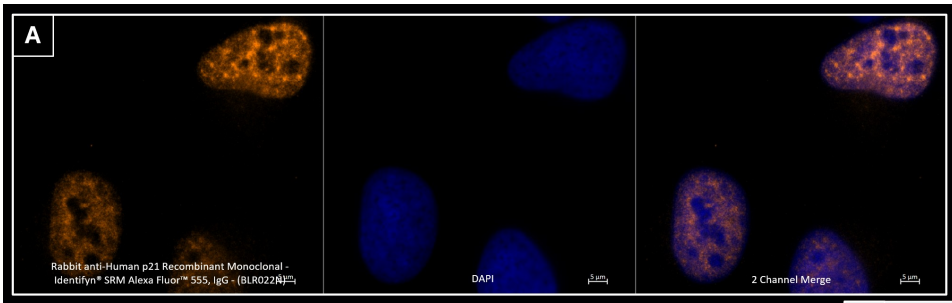
NONE

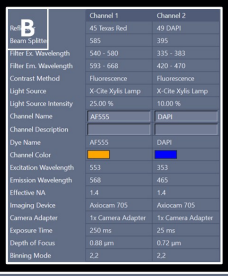
Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000079
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6EE3B8C75E9934D2CA63A1331367D0E9933EFD1AE37047E5750F272FA36BE826
Method PDF SHA-256	582763DF4DEF82FBD13DA65B25E10C3D13091B5696A6F0B9E12E4118B39643FC

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



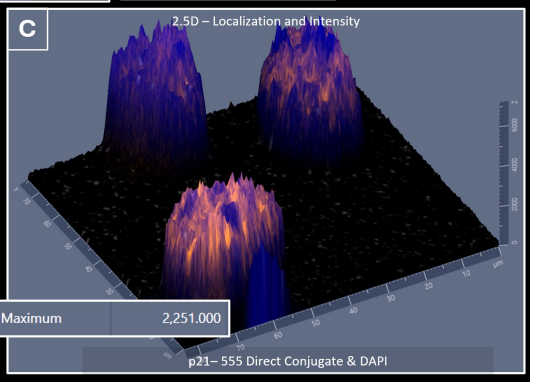


Widefield

Panel A depicts widefield fluorescence microscopy of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N). The left panel shows direct p21-associated fluorescence localization displayed in orange; the middle panel shows DAPI nuclear counterstaining in blue; and the right panel shows the merged image. Strong nuclear-associated fluorescence localization and diffuse nuclear enrichment of p21 signal were observed, with minimal background interference, demonstrating effective target localization and preservation of signal integrity following direct fluorophore conjugation. Signal distribution and localization characteristics remained highly consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) (Cat ID# - PA-RR 000009) stepdown dataset, supporting preservation of target specificity and biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents the Zeiss widefield acquisition parameters utilized during image collection. Imaging of the AF555 channel was performed using the Texas Red filter set with the X-Cite Xylis Lamp operating at 25.00% intensity and an exposure time of 250 ms, while DAPI acquisition was performed using 10.00% lamp intensity with a 25 ms exposure time. These acquisition settings demonstrated strong fluorescence signal generation and preservation of nuclear-associated p21 localization under standard widefield fluorescence microscopy conditions. The ability to generate robust target-associated fluorescence enrichment under moderate excitation and exposure settings further supports the robustness of direct fluorophore conjugation and preservation of biologically coherent localization behavior following labeling with Identifyn® SRM Alexa Fluor™ 555.

Panel C shows a Zeiss ZEN 2.5D fluorescence intensity representation generated from the acquired widefield dataset. Intensity mapping demonstrated strong nuclear-associated localization and broad p21-associated fluorescence enrichment throughout DAPI-positive nuclei. Quantitative analysis performed using the Zeiss ZEN histogram tool demonstrated a maximum fluorescence intensity of 2,251.000 A.U., supporting robust fluorophore conjugation and strong signal generation under standard widefield fluorescence microscopy acquisition conditions. Collectively, these findings demonstrate that Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) maintained strong fluorescence performance, nuclear specificity, and compatibility with conventional widefield fluorescence microscopy workflows following direct fluorophore conjugation.



Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N)
Cat ID# - DC 000079

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555
METADATA VALUES	40

STRUCTURED ACQUISITION METADATA / WIDEFIELD

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image Size (Pixels)	793 X 691
Image Size (Scaled)	83.57 µm X 75.68 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	45 Texas Red | 49 DAPI
Beam Splitter	585 | 395
Filter Excitation Wavelength	540 - 580 | 335 - 383
Filter Emission Wavelength	593 - 668 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 25.00% | X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	250 ms | 25 ms
Excitation Wavelength	553 | 353
Emission Wavelength	568 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X | 1x Camera Adapter
Depth of Focus	0.88 µm | 0.72 µm
Binning Mode	2,2
Render Mode	Surface
Grid distance	5
Angle X	53
Angle Y	-151
Z Scaling	1.0
Ambient	50
Specular	20
Shininess	50
Light Height	2.0

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N)
Cat ID# - DC 000079

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

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 12 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 - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image Size (Pixels) = 793 X 691
Image Size (Scaled) = 83.57 µm X 75.68 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 @ 25.00%
Exposure Time = 250 ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.88 µm
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 @ 10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x Camera Adapter
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The left panel shows Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N). The middle panel shows DAPI nuclear counterstaining. The right panel shows the merged image, demonstrating strong nuclear-associated p21 localization and diffuse nuclear fluorescence enrichment, supporting robust conjugation performance and preservation of target-specific fluorescence signal under widefield fluorescence microscopy conditions.

ZEN Acquisition Info Tab - Panel B

Shown is the acquisition interface for the Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) widefield dataset collected using the X-Cite Xylis Lamp and Zeiss Axiocam 705 camera. Imaging of the p21-associated 555 channel was performed using 25% X-Cite Xylis Lamp intensity with a 250 ms exposure time on the Axiocam 705, while DAPI acquisition was performed using 10% lamp intensity with a 25 ms exposure time. These acquisition conditions demonstrated robust fluorescence signal generation and strong nuclear-associated p21 localization under standard widefield excitation and collection settings. The ability to generate strong diffuse nuclear p21-associated fluorescence enrichment and target-specific signal under these moderate acquisition parameters further supports the robustness of direct conjugation and preservation of biologically coherent localization behavior following labeling with Identifyn® SRM Alexa Fluor™ 555.

2.5D Image Processing - Panel C

2.5D Display

Render Mode = Surface
Grid distance = 5
Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 53
Angle Y = -151
Z Scaling = 1.0
Ambient = 50
Specular = 20
Shininess = 50
Light Height = 2.0

ZEN Acquisition Histogram Data - Panel C

Shown is the maximum fluorescence intensity measurement obtained from the Zeiss ZEN Histogram tool for the Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) direct conjugate, demonstrating a peak fluorescence intensity of 2,251.000 A.U., supporting robust fluorophore conjugation and strong signal generation under standard widefield fluorescence microscopy acquisition conditions.

Summary

Panel A depicts widefield fluorescence microscopy of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N). The left panel shows direct p21-associated fluorescence localization displayed in orange; the middle panel shows DAPI nuclear counterstaining in blue; and the right panel shows the merged image. Strong nuclear-associated fluorescence localization and diffuse nuclear enrichment of p21 signal were observed, with minimal background interference, demonstrating effective target localization and preservation of signal integrity following direct fluorophore conjugation. Signal distribution and localization characteristics remained highly consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) (Cat ID# - PA-RR 000009) stepdown dataset, supporting preservation of target specificity and biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents the Zeiss widefield acquisition parameters utilized during image collection. Imaging of the AF555 channel was performed using the Texas Red filter set with the X-Cite Xylis Lamp operating at 25.00% intensity and an exposure time of 250 ms, while DAPI acquisition was performed using 10.00% lamp intensity with a 25 ms exposure time. These acquisition settings demonstrated strong fluorescence signal generation and preservation of nuclear-associated p21 localization under standard widefield fluorescence microscopy conditions. The ability to generate robust target-associated fluorescence enrichment under moderate excitation and exposure settings further supports the robustness of direct fluorophore conjugation and preservation of biologically coherent localization behavior following labeling with Identifyn® SRM Alexa Fluor™ 555.

Panel C shows a Zeiss ZEN 2.5D fluorescence intensity representation generated from the acquired widefield dataset. Intensity mapping demonstrated strong nuclear-associated localization and broad p21-associated fluorescence enrichment throughout DAPI-positive nuclei. Quantitative analysis performed using the Zeiss ZEN histogram tool demonstrated a maximum fluorescence intensity of 2,251.000 A.U., supporting robust fluorophore conjugation and strong signal generation under standard widefield fluorescence microscopy acquisition conditions. Collectively, these findings demonstrate that Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) maintained strong fluorescence performance, nuclear specificity, and compatibility with conventional widefield fluorescence microscopy workflows following direct fluorophore conjugation.

Image Correction

NONE

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

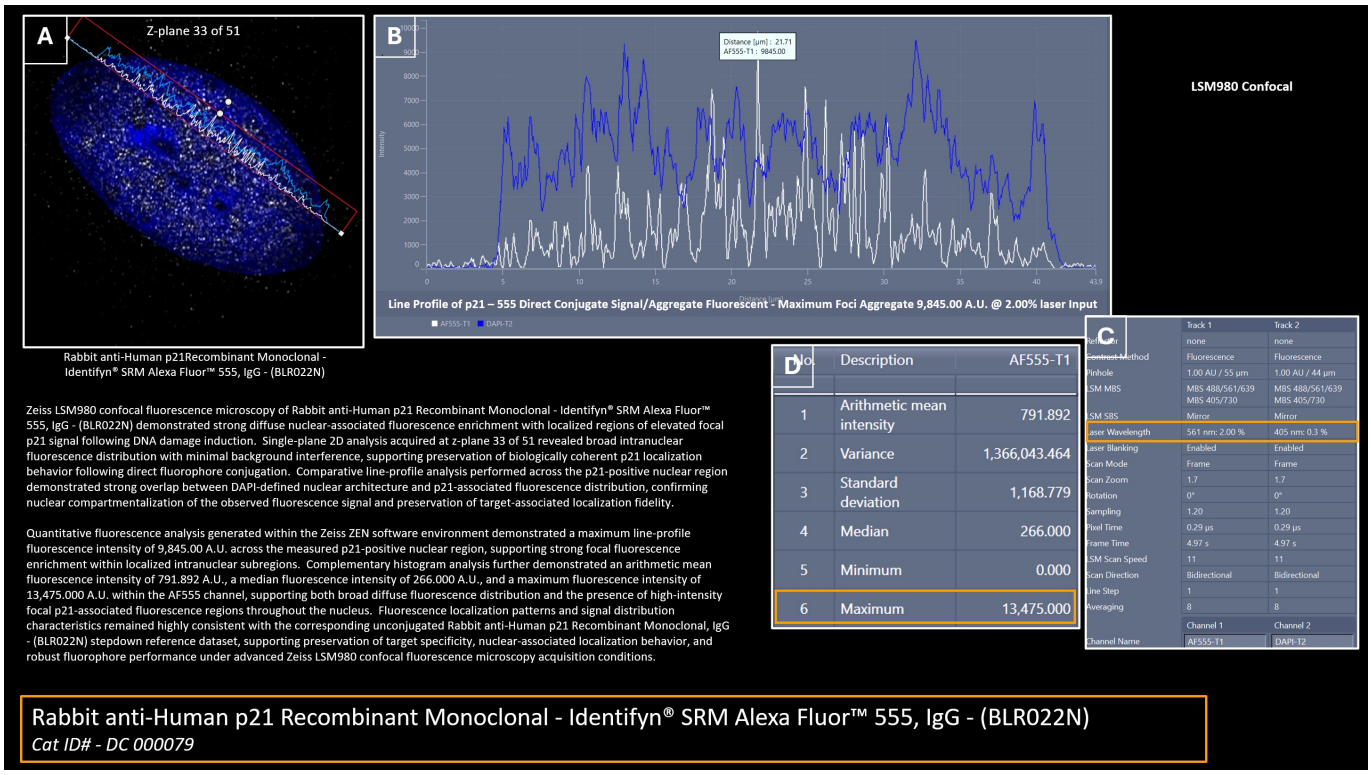
SOURCE PROVENANCE

Catalog	DC-000079
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	40

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	48152473CEB943A014A75E1D3A7DB3A93F0A67A2D051D505B092F27B86FBACD5
Method PDF SHA-256	35570C4CA3280CD72764CAF0B9CB3687AB966FEA09E324275E33497DBAFB8C69

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Rabbit anti-Human p21Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N)

Zeiss LSM980 confocal fluorescence microscopy of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) demonstrated strong diffuse nuclear-associated fluorescence enrichment with localized regions of elevated focal p21 signal following DNA damage induction. Single-plane 2D analysis acquired at z-plane 33 of 51 revealed broad intranuclear fluorescence distribution with minimal background interference, supporting preservation of biologically coherent p21 localization behavior following direct fluorophore conjugation. Comparative line-profile analysis performed across the p21-positive nuclear region demonstrated strong overlap between DAPI-defined nuclear architecture and p21-associated fluorescence distribution, confirming nuclear compartmentalization of the observed fluorescence signal and preservation of target-associated localization fidelity.

Quantitative fluorescence analysis generated within the Zeiss ZEN software environment demonstrated a maximum line-profile fluorescence intensity of 9,845.00 A.U. across the measured p21-positive nuclear region, supporting strong focal fluorescence enrichment within localized intranuclear subregions. Complementary histogram analysis further demonstrated an arithmetic mean fluorescence intensity of 791.892 A.U., a median fluorescence intensity of 266.000 A.U., and a maximum fluorescence intensity of 13,475.000 A.U. within the AF555 channel, supporting both broad diffuse fluorescence distribution and the presence of high-intensity focal p21-associated fluorescence regions throughout the nucleus. Fluorescence localization patterns and signal distribution characteristics remained highly consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) stepdown reference dataset, supporting preservation of target specificity, nuclear-associated localization behavior, and robust fluorophore performance under advanced Zeiss LSM980 confocal fluorescence microscopy acquisition conditions.

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N)
Cat ID# - DC 000079

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	45

STRUCTURED ACQUISITION METADATA / CONFOCAL

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	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:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	51 Slices (1.50 µm)
Channels	2
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	754 X 734
Image Size (Scaled)	44.35 µm X 43.17 µm
Bit Depth	16 Bit
Image Center Position	X: 93.56 mm, Y: 52.68 mm
Stage Position	X: 93.56 mm, Y: 52.68 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 55 µm | 1.00 AU / 44 µm
LSM MBS	MBS 488/561/639 & 405/730
LSM SBS	Mirror
Laser Wavelength	561 nm @ 2.00% | 405 nm @ 0.3%
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	553 | 353
Emission Wavelength	568 | 465
Effective NA	1.4
Detection Wavelength	550 - 600 | 430 - 490
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	600 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.9 (3D, Auto) | Filter: 6.8 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 43.9), Y (Min 0.0 and Max 10337)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N)
Cat ID# - DC 000079

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

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 12 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 - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

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) - Panel A, 2-Dimensional view at Z-plane 33 of 51, Line Profile Panel B

Z-Stack = 51 Slices (1.50 µm)

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 754 X 734
Image Size (Scaled) = 44.35 µm X 43.17 µm
Bit Depth = 16 Bit
Image Center Position = X: 93.56 mm, Y: 52.68 mm
Stage Position = X: 93.56 mm, Y: 52.68 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 55 µm
LSM MBS = MBS 488/561/639 & 405/730
LSM SBS = Mirror
Laser Wavelength = 561 nm @ 2.00%
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 = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 550 - 600
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.9 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 µm
LSM MBS = MBS 488/561/639 & 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @ 0.3%
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 = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430 - 490
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.8 (3D, Auto)

2- Dimensional View - Panel A

Panel A depicts a single-plane 2D Zeiss LSM980 confocal fluorescence microscopy image of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) acquired at z-plane 33 of 51 following DNA damage induction. The p21-associated fluorescence signal is pseudocolored white to enhance visualization of fluorescence distribution, aggregate formation, and intranuclear localization characteristics. A line profile was drawn diagonally across the nucleus to assess fluorescence intensity distribution and signal continuity throughout the p21-positive nuclear region, which is subsequently quantified in Panel B.

Profile View Display - Panel B

To further evaluate fluorescence localization characteristics and nuclear compartmentalization behavior, a comparative line-profile analysis was performed across the p21-positive nuclear region identified in Panel A. DAPI fluorescence intensity was included in the profile analysis to provide a direct comparison between the p21-associated fluorescence distribution and the corresponding nuclear compartment. Comparative fluorescence tracing demonstrated strong overlap between DAPI-defined nuclear architecture and diffuse p21-associated fluorescence enrichment, supporting preservation of biologically coherent nuclear localization behavior following direct fluorophore conjugation. Quantitative fluorescence analysis further demonstrated a maximum aggregate-associated p21 fluorescence intensity of 9,845.00 A.U. across the measured profile, supporting robust signal generation and preservation of strong target-associated fluorescence enrichment under Zeiss LSM980 confocal microscopy acquisition conditions.

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

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 43.9), Y (Min 0.0 and Max 10337)

Measured at Z-plane 33 of 51

ZEN Acquisition Info Tab - Panel C

Shown are the Zeiss LSM980 confocal acquisition parameters utilized during image collection for Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N). Imaging of the AF555 channel was performed using the 561 nm laser operating at 2.00% power, while DAPI acquisition was performed using the 405 nm laser operating at 0.3% power. Acquisition was performed in frame-scan mode with a scan zoom of 1.7, bidirectional scanning, scan speed 11, averaging set to 8, and a pixel time of 0.29 µs. Sampling was maintained at 1.20 with a frame acquisition time of 4.97 seconds. These acquisition parameters demonstrated robust fluorescence signal generation, strong aggregate-associated p21 localization, and preservation of biologically coherent fluorescence distribution characteristics under standard Zeiss LSM980 confocal microscopy acquisition conditions.

Zen Histogram Data - Panel D

Shown is the Zeiss ZEN histogram analysis for Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N), demonstrating an arithmetic mean fluorescence intensity of 791.892 A.U., a median fluorescence intensity of 266.000 A.U., and a maximum fluorescence intensity of 13,475.000 A.U. within the AF555 channel. The elevated maximum fluorescence intensity relative to the arithmetic mean supports the presence of strong aggregate-associated and focal intranuclear p21 fluorescence enrichment within localized nuclear subregions, while the broader fluorescence distribution profile remains consistent with diffuse nuclear-associated p21 localization behavior observed throughout the confocal dataset. The observed fluorescence variance and standard deviation further support biologically heterogeneous intranuclear fluorescence distribution patterns associated with p21 recruitment and accumulation following DNA damage induction.

Summary

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) demonstrated strong fluorescence performance, robust nuclear-associated signal generation, and preservation of biologically coherent p21 localization characteristics under advanced Zeiss LSM980 confocal fluorescence microscopy acquisition conditions following direct fluorophore conjugation. Confocal imaging revealed broad, diffuse intranuclear fluorescence enrichment with localized regions of aggregate-associated signal enhancement throughout DAPI-positive nuclear compartments following DNA damage induction, consistent with the established biological roles of p21 in the DNA damage response, cell-cycle regulation, and nuclear repair-associated signaling pathways. Fluorescence localization patterns remained highly consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) (Cat ID# - PA-RR 000009) stepdown dataset, supporting preservation of target specificity and biologically coherent nuclear localization behavior following direct conjugation with Identifyn® SRM Alexa Fluor™ 555.

Quantitative fluorescence assessment demonstrated strong signal generation across multiple analytical modalities within the Zeiss ZEN software environment. Comparative line-profile analysis showed strong overlap between the DAPI-defined nuclear architecture and the p21-associated fluorescence distribution, confirming nuclear compartmentalization of the observed signal and the preservation of biologically relevant localization characteristics. The maximum aggregate-associated fluorescence intensity measured across the analyzed line profile reached 9,845.00 A.U., indicating strong focal fluorescence enrichment within localized intranuclear subregions. Complementary histogram analysis further demonstrated an arithmetic mean fluorescence intensity of 791.892 A.U. and a maximum fluorescence intensity of 13,475.000 A.U. in the AF555 channel, supporting both a broad, diffuse fluorescence distribution and the presence of high-intensity focal fluorescence regions throughout the nucleus.

Elevated variance and standard deviation measurements further supported heterogeneous but biologically coherent intranuclear fluorescence enrichment patterns associated with p21 recruitment following DNA damage induction.

Collectively, the confocal dataset demonstrated that Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) maintained strong fluorophore performance, preserved target specificity, and generated biologically coherent fluorescence localization patterns compatible with high-resolution confocal fluorescence microscopy workflows. The ability to generate robust fluorescence intensity, maintain nuclear-associated localization fidelity, and preserve consistency relative to the unconjugated stepdown reference dataset further supports the effectiveness of the Identifyn® SRM direct conjugation strategy under advanced confocal imaging conditions.

Image Correction

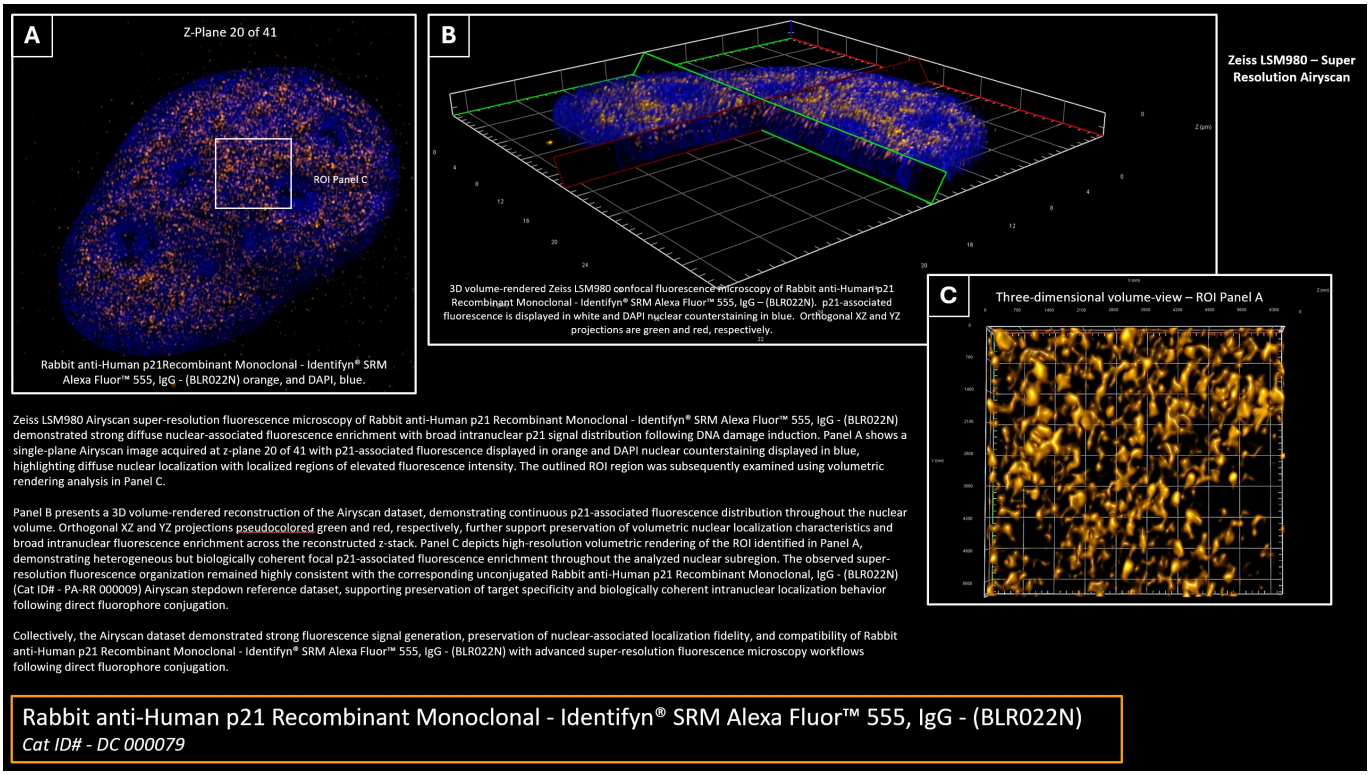
NONE

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000079
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	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:
Structured source metadata values	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	15D6B6013CED04603CD7FE942E45705D8E13DCBC498F937E45B09D0D28C2879A
Method PDF SHA-256	872AE5A863083CD10DD2DB3D9A20647484D881A98CE0FE362B73300582D6C7BA

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	60

STRUCTURED ACQUISITION METADATA / AIRYSCAN

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	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:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	41 Slices (1.20 µm)
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	1005 X 940 | 186 X 168
Image Size (Scaled)	35.47 µm X 33.18 µm | 6.56 µm X 5.93 µm
Bit Depth	16 Bit
Image Center Position	X: 94.14 mm, Y: 53.83 mm
Stage Position	X: 94.14 mm, Y: 53.83 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 309 µm | 5.00 AU / 219 µm
LSM MBS	MBS 488/561 & MBS 405/730
LSM SBS	SBS LP 525 | SBS SP 505
Laser Wavelength	561 nm @ 2.00% | 405 nm @ 0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	2.00
Pixel Time	1.10 µs
Frame Time	18.77 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	553 | 353
Emission Wavelength	568 | 465
Effective NA	1.4
Detection Wavelength	527-735 | 420-480 / 495 - 499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	850 V | 800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution: 10.0 (3D, Auto) | SuperResolution: 9.3 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels | Threshold 4%, Ramp 96%, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, 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) | View Angle 35°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
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	2% | 0%
Clip Y	-45° | 45°
Clip Z	0°
Y-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N)
Cat ID# - DC 000079

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

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 12 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 - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

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) - Panel A, 2-Dimensional view at Z-plane 20 of 41, 3D Volume ViewPanel B

Z-Stack = 41 Slices (1.20 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 1005 X 940
Image Size (Scaled) = 35.47 µm X 33.18 µm
Bit Depth = 16 Bit
Image Center Position = X: 94.14 mm, Y: 53.83 mm
Stage Position = X: 94.14 mm, Y: 53.83 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - 3D Volume ViewPanel C

Z-Stack = 41 Slices (1.20 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 186 X 168
Image Size (Scaled) = 6.56 µm X 5.93 µm
Bit Depth = 16 Bit
Image Center Position = X: 94.14 mm, Y: 53.83 mm
Stage Position = X: 94.14 mm, Y: 53.83 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 309 µm
LSM MBS = MBS 488/561 & MBS 405/730
LSM SBS = SBS LP 525
Laser Wavelength = 561 nm @ 2.00%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10 µs
Frame Time = 18.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 527-735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution: 10.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 219 µm
LSM MBS = MBS 488/561 & MBS 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @ 0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10 µs
Frame Time = 18.77 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: 9.3 (3D, Auto)

2-Dimensional View - Panel A

Panel A depicts Zeiss LSM980 Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) acquired at z-plane 20 of 41 following DNA damage induction. p21-associated fluorescence is displayed in orange, and DAPI nuclear counterstaining is blue. Broad diffuse nuclear-associated fluorescence enrichment with localized regions of elevated focal p21 signal was observed throughout the nucleus with minimal background interference, supporting preservation of biologically coherent intranuclear localization behavior following direct fluorophore conjugation. The boxed ROI region was subsequently utilized for high-resolution volumetric rendering analysis in Panel C.

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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Processing - Panel B

Clipping planes are introduced in Panel B to highlight Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) within the nuclear compartment through orthogonal wedge-cut visualization. Portions of the volumetric dataset were selectively removed across the X-Z and Y-Z planes to facilitate assessment of intranuclear p21-associated fluorescence distribution, diffuse nuclear enrichment, and volumetric signal organization throughout the reconstructed Airyscan dataset.

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 = 2%
Clip Y = -45°
Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 0%
Clip Y = 45°
Clip Z = 0°

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 4%, Ramp 96%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Zeiss LSM980 Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) demonstrated strong diffuse nuclear-associated fluorescence enrichment with broad intranuclear p21 signal distribution following DNA damage induction. Panel A shows a single-plane Airyscan image acquired at z-plane 20 of 41 with p21-associated fluorescence displayed in orange and DAPI nuclear counterstaining displayed in blue, highlighting diffuse nuclear localization with localized regions of elevated fluorescence intensity. The outlined ROI region was subsequently examined using volumetric rendering analysis in Panel C.

Panel B presents a 3D volume-rendered reconstruction of the Airyscan dataset, demonstrating a continuous distribution of p21-associated fluorescence throughout the nuclear volume. Orthogonal XZ and YZ projections, pseudocolored green and red, respectively, further support preservation of volumetric nuclear localization characteristics and broad intranuclear fluorescence enrichment across the reconstructed z-stack. Panel C depicts a high-resolution volumetric rendering of the ROI identified in Panel A, demonstrating heterogeneous but biologically coherent focal p21-associated fluorescence enrichment throughout the analyzed nuclear subregion. The observed super-resolution fluorescence organization remained highly consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) (Cat ID# - PA-RR 000009) Airyscan stepdown reference dataset, supporting preservation of target specificity and biologically coherent intranuclear localization behavior following direct fluorophore conjugation.

Collectively, the Airyscan dataset demonstrated strong fluorescence signal generation, preservation of nuclear-associated localization fidelity, and compatibility of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR022N) with advanced super-resolution fluorescence microscopy workflows following direct fluorophore conjugation.

Image Correction

NONE

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000079
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	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:
Structured source metadata values	60
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D4534AFF6D3DCF3187E4180EC7A81497D41169C157AC140DC3DDAE501278913A
Method PDF SHA-256	B75AF32E26B1415A7F680A953D665015F1030A5C59E95D3F4F8C456B9045D19F