BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

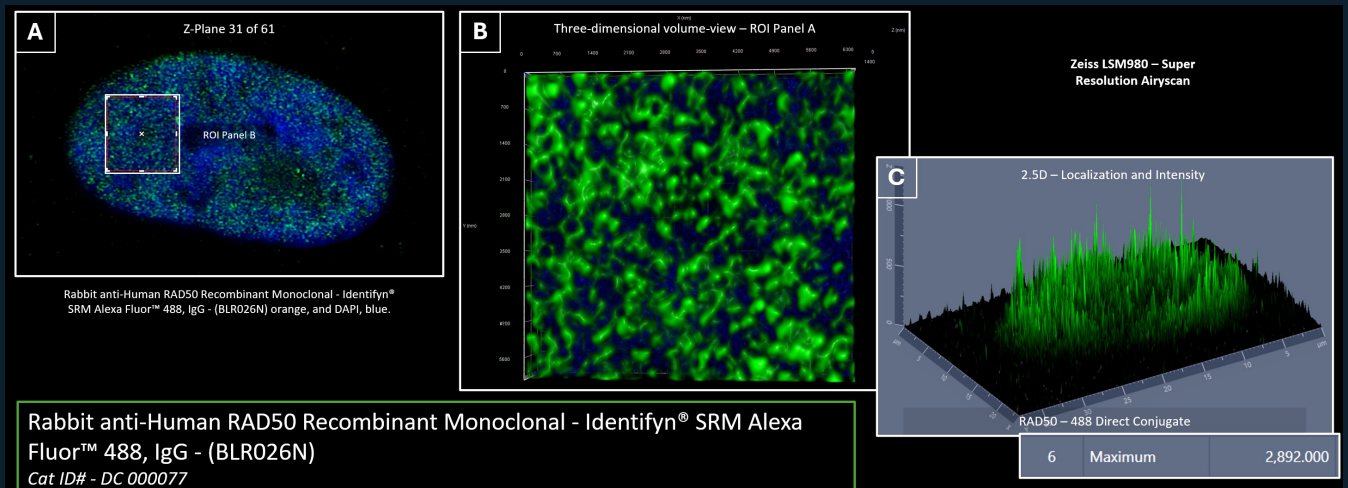
RAD50

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488

WESTERN BLOT -> WIDEFIELD -> CONFOCAL -> AIRYSCAN

4	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) orange, and DAPI, blue.

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N)
Cat ID# - DC 000077

Panel A depicts Zeiss LSM980 Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) at Z-plane 31 of 61 following DNA damage induction. Strong nuclear-associated fluorescence localization and punctate RAD50-associated signal enrichment were observed throughout the nucleus, demonstrating effective target localization and preservation of signal integrity following direct fluorophore conjugation. The highlighted ROI selected for further volumetric analysis demonstrated dense focal fluorescence accumulation patterns consistent with DNA damage-associated repair foci formation. Signal distribution and localization characteristics remained highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, supporting preservation of target specificity and biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents a three-dimensional volumetric rendering generated from the ROI identified in Panel A. Airyscan super-resolution imaging demonstrated pronounced RAD50-associated fluorescence localization and dense focal aggregate distribution throughout the selected nuclear region, supporting preservation of structural localization detail and enhanced visualization of DNA damage-associated repair complex accumulation behavior under super-resolution acquisition conditions. However, as consistently observed across RAD50 super-resolution workflows, Airyscan imaging can conflate individual RAD50-associated repair foci and aggregate structures due to the modality's inherent resolution limitations. While Airyscan provides substantial improvement over conventional confocal fluorescence microscopy, definitive structural interpretation and discrete aggregate confirmation are only achieved following STORM super-resolution analysis.

Panel C shows a Zeiss ZEN 2.5D fluorescence intensity representation generated from the Airyscan dataset. Quantitative fluorescence analysis demonstrated a maximum fluorescence intensity of 2,892.000 A.U., supporting robust fluorophore conjugation and strong signal generation under Airyscan super-resolution microscopy conditions. Imaging of the AF488 channel was performed using the 488 nm laser at 2.0% power, demonstrating that strong RAD50-associated fluorescence localization and focal aggregate enrichment were achieved under moderate excitation conditions, without reliance on excessive laser power. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) maintained strong fluorescence performance, focal DNA damage-associated localization characteristics, and compatibility with high-resolution Airyscan fluorescence microscopy workflows following direct fluorophore conjugation.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / AIRYSCAN

DC-000077 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

UNPUBLISHED / PENDING SCIENTIST REVIEW / NO PUBLICATION AUTHORIZED

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 RAD50, 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-000077 record contains the applicable NIR sequence: Western blot; Widefield; Confocal; Airyscan. The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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 / NIR-BOUNDED PARTIAL STEPDOWN

The preserved DC-000077 record contains the applicable NIR sequence: Western blot; Widefield; Confocal; Airyscan. The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 555	2; 38 eGFP; 49 DAPI; 450 - 490; 335 - 383; 500 - 550; 420 - 470; 493
Confocal	405/DAPI; 488	2; None; 488 nm @ 1.50%; 405 nm @ 0.2%; 493; 353; 517; 465
Airyscan	405/DAPI; 488; 750	2; None; 488 nm @ 2.0%; 405 nm @ 0.2%; 493; 353; 517; 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 488.

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): 770 X 638; Image Size (Pixels): 770 X 638; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 300 ms; 25 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 30.00%; X-Cite Xylis Lamp Intensity @ 10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 39.

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; 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

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: 81 Slices (2.40 µm); Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1085 X 916; Image Size (Pixels): 1085 X 916; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.28 µs; Frame Time: 5.28 s. Illumination: Laser Wavelength: 488 nm @ 1.50%; 405 nm @ 0.2%. Optical/scan: Pinhole: 1.00 AU / 51 µm; 1.00 AU / 43 µm; Scan Zoom: 1.6; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.1 (3D, Auto); Filter: 6.8 (3D, Auto). Structured source metadata values: 46.

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.

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: 61 Slices (1.80 µm); Channels: 2; Scaling (Per Pixel): 35.29 nm X 35.29 nm X 30.00 nm; Image size (Pixels): 663 X 1072; 183 X 171; Image Size (Pixels): 663 X 1072; 183 X 171; 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: 488 nm @ 2.0%; 405 nm @ 0.2%. Optical/scan: Pinhole: 5.00 AU / 250 µm; 5.00 AU / 214 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); Background: Black; AiryScan Mode: SuperResolution 9.2 (3D, Auto); SuperResolution 9.4 (3D, Auto). Structured source metadata values: 58.

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; 750.

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-000077 record contains the applicable NIR sequence: Western blot; Widefield; Confocal; Airyscan. The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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): 35.29 nm X 35.29 nm X 30.00 nm -> 0.03529 µm X 0.03529 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)=663 X 1072; Image Size (Scaled)=23.40 µm X 37.84 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

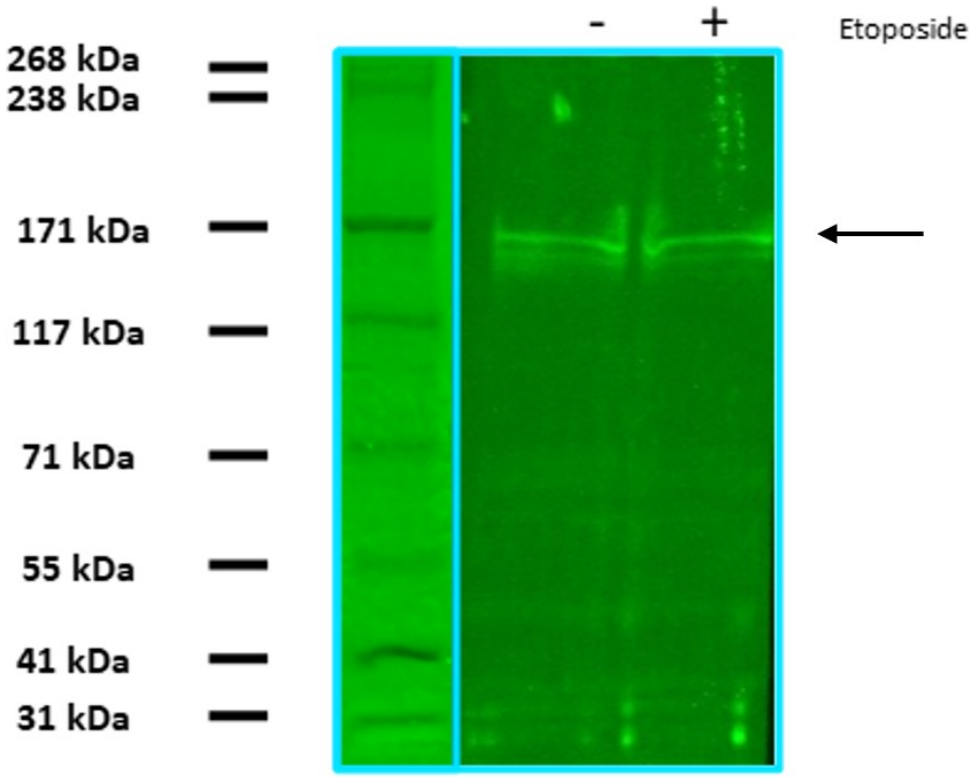
This dossier preserves the complete available evidence progression for DC-000077. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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	488
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	488 nm
Emission Filter	513 ± 17 nm
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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N)
Cat ID# - DC 000077

Expected MW: 154 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 on NuPAGE™ Tris-Acetate Mini Protein Gels (3-8%, 1.0 mm) in a Mini Gel Tank. Thermo Fisher™ HiMark™ Pre-stained Protein Standard 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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) 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 = 488 nm
Emission Filter = 513 ± 17 nm
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

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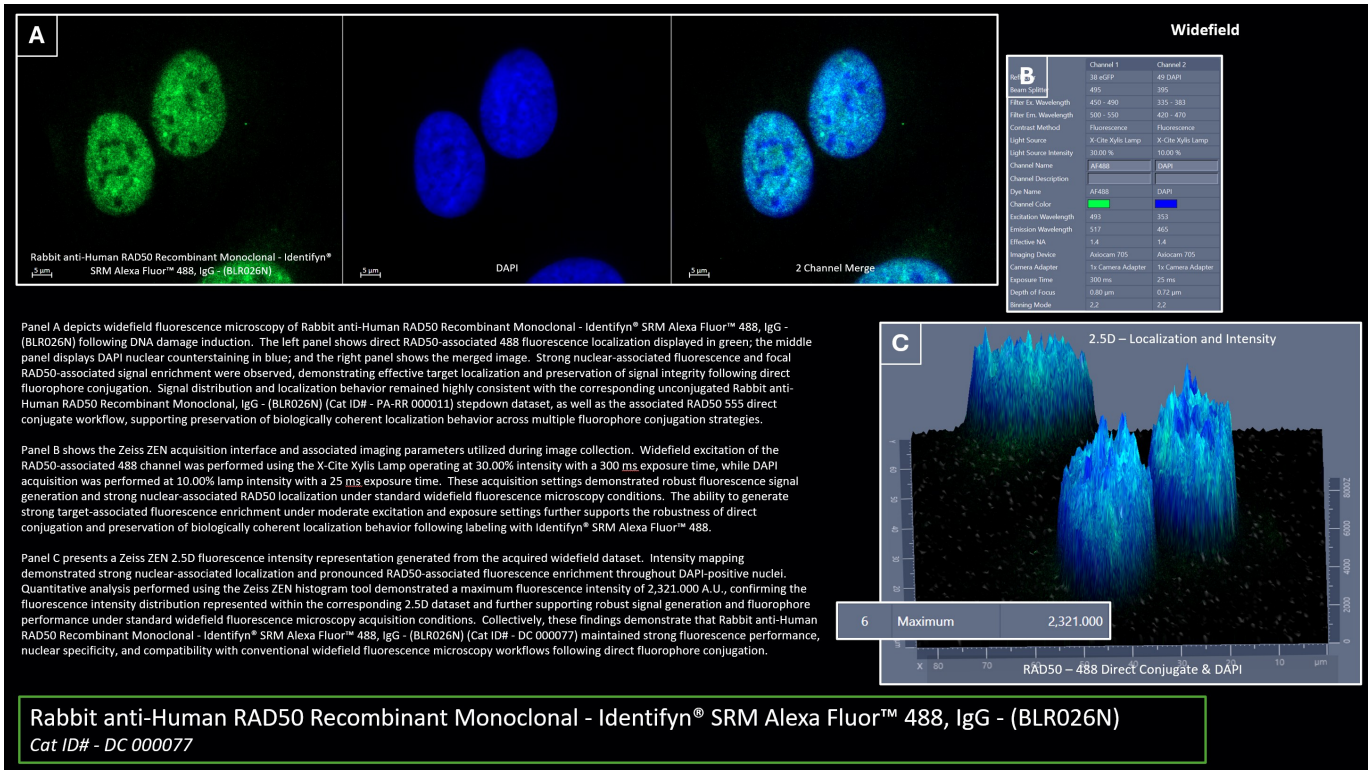
SOURCE PROVENANCE

Catalog	DC-000077
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	E28BA5CE23C6A8BAF05ADDA8A8B41439A036271416323B067B2B01D386AA1197
Method PDF SHA-256	992EB7D5F07558A57947569F76C93AF188D1DBB346E59FDE910D039B0C9FEBA3

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A depicts widefield fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) following DNA damage induction. The left panel shows direct RAD50-associated 488 fluorescence localization displayed in green; the middle panel displays DAPI nuclear counterstaining in blue; and the right panel shows the merged image. Strong nuclear-associated fluorescence and focal RAD50-associated signal enrichment were observed, demonstrating effective target localization and preservation of signal integrity following direct fluorophore conjugation. Signal distribution and localization behavior remained highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, as well as the associated RAD50 555 direct conjugate workflow, supporting preservation of biologically coherent localization behavior across multiple fluorophore conjugation strategies.

Panel B shows the Zeiss ZEN acquisition interface and associated imaging parameters utilized during image collection. Widefield excitation of the RAD50-associated 488 channel was performed using the X-Cite Xylis Lamp operating at 30.00% intensity with a 300 ms exposure time, while DAPI acquisition was performed at 10.00% lamp intensity with a 25 ms exposure time. These acquisition settings demonstrated robust fluorescence signal generation and strong nuclear-associated RAD50 localization under standard widefield fluorescence microscopy conditions. The ability to generate strong target-associated fluorescence enrichment under moderate excitation and exposure settings further supports the robustness of direct conjugation and preservation of biologically coherent localization behavior following labeling with Identifyn® SRM Alexa Fluor™ 488.

Panel C presents a Zeiss ZEN 2.5D fluorescence intensity representation generated from the acquired widefield dataset. Intensity mapping demonstrated strong nuclear-associated localization and pronounced RAD50-associated fluorescence enrichment throughout DAPI-positive nuclei. Quantitative analysis performed using the Zeiss ZEN histogram tool demonstrated a maximum fluorescence intensity of 2,321.000 A.U., confirming the fluorescence intensity distribution represented within the corresponding 2.5D dataset and further supporting robust signal generation and fluorophore performance under standard widefield fluorescence microscopy acquisition conditions. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) (Cat ID# - DC 000077) maintained strong fluorescence performance, nuclear specificity, and compatibility with conventional widefield fluorescence microscopy workflows following direct fluorophore conjugation.

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N)
Cat ID# - DC 000077

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	39

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)	770 X 638
Image Size (Scaled)	84.33 µm X 69.98 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	38 eGFP | 49 DAPI
Beam Splitter	495 | 395
Filter Excitation Wavelength	450 - 490 | 335 - 383
Filter Emission Wavelength	500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 30.00% | X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	300 ms | 25 ms
Excitation Wavelength	493 | 353
Emission Wavelength	517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1x Camera Adapter
Depth of Focus	0.80 µm | 0.72 µm
Binning Mode	2,2
Render Mode	Surface
Grid distance	5
Angle X	52
Angle Y	-179
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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N)
Cat ID# - DC 000077

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for the identification and processing of DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N), 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) = 770 X 638
Image Size (Scaled) = 84.33 µm X 69.98 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

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 30.00%
Exposure Time = 300 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x Camera Adapter
Depth of Focus = 0.80 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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N). The middle panel shows DAPI nuclear counterstaining. The right panel shows the merged image, demonstrating strong nuclear localization and focal RAD50-associated fluorescence enrichment following DNA damage induction, 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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) widefield dataset collected using the X-Cite Xylis Lamp and Zeiss Axiocam 705 camera. Imaging of the RAD50-associated 488 channel was performed using 30% X-Cite Xylis Lamp intensity with a 300 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 RAD50 localization under standard widefield excitation and collection settings. The ability to generate strong focal RAD50-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™ 488.

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 = 52
Angle Y = -179
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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) direct conjugate, demonstrating a peak fluorescence intensity of 2,321.000 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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) following DNA damage induction. The left panel shows direct RAD50-associated 488 fluorescence localization displayed in green; the middle panel displays DAPI nuclear counterstaining in blue; and the right panel shows the merged image. Strong nuclear-associated fluorescence and focal RAD50-associated signal enrichment were observed, demonstrating effective target localization and preservation of signal integrity following direct fluorophore conjugation. Signal distribution and localization behavior remained highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, as well as the associated RAD50 555 direct conjugate workflow, supporting preservation of biologically coherent localization behavior across multiple fluorophore conjugation strategies.

Panel B shows the Zeiss ZEN acquisition interface and associated imaging parameters utilized during image collection. Widefield excitation of the RAD50-associated 488 channel was performed using the X-Cite Xylis Lamp operating at 30.00% intensity with a 300 ms exposure time, while DAPI acquisition was performed at 10.00% lamp

intensity with a 25 ms exposure time. These acquisition settings demonstrated robust fluorescence signal generation and strong nuclear-associated RAD50 localization under standard widefield fluorescence microscopy conditions. The ability to generate strong target-associated fluorescence enrichment under moderate excitation and exposure settings further supports the robustness of direct conjugation and preservation of biologically coherent localization behavior following labeling with Identifyn® SRM Alexa Fluor™ 488.

Panel C presents a Zeiss ZEN 2.5D fluorescence intensity representation generated from the acquired widefield dataset. Intensity mapping demonstrated strong nuclear-associated localization and pronounced RAD50-associated fluorescence enrichment throughout DAPI-positive nuclei. Quantitative analysis performed using the Zeiss ZEN histogram tool demonstrated a maximum fluorescence intensity of 2,321.000 A.U., confirming the fluorescence intensity distribution represented within the corresponding 2.5D dataset and further supporting robust signal generation and fluorophore performance under standard widefield fluorescence microscopy acquisition conditions. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) (Cat ID# - DC 000077) 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

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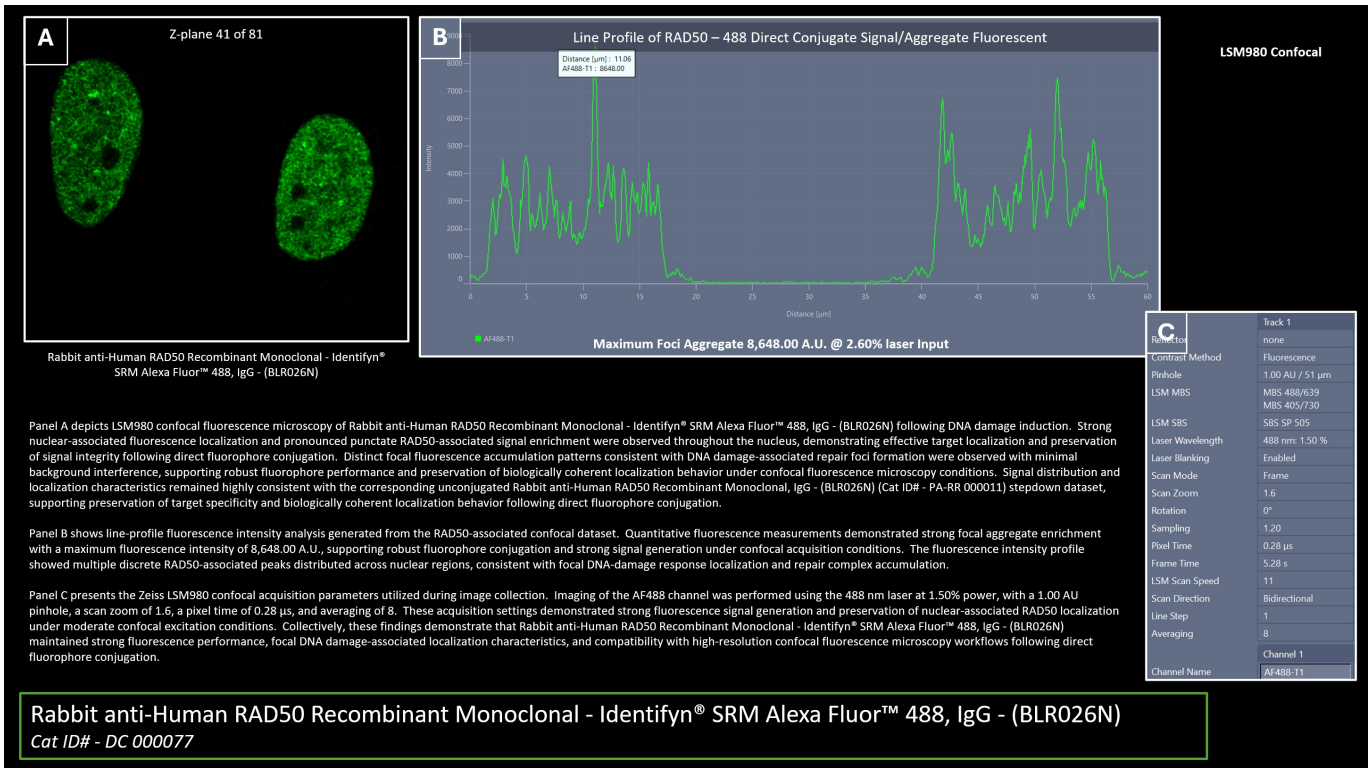
SOURCE PROVENANCE

Catalog	DC-000077
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	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	EE1F4A49EEEBD45D3AC88C9728D76CF2B68A7495A59E3AF1724DB16E8109B66F
Method PDF SHA-256	7D694F01127B4FE77A2A406AFE09845371000B308728A82DBCD8549DDE9E0315

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N)

Panel A depicts LSM980 confocal fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) following DNA damage induction. Strong nuclear-associated fluorescence localization and pronounced punctate RAD50-associated signal enrichment were observed throughout the nucleus, demonstrating effective target localization and preservation of signal integrity following direct fluorophore conjugation. Distinct focal fluorescence accumulation patterns consistent with DNA damage-associated repair foci formation were observed with minimal background interference, supporting robust fluorophore performance and preservation of biologically coherent localization behavior under confocal fluorescence microscopy conditions. Signal distribution and localization characteristics remained highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, supporting preservation of target specificity and biologically coherent localization behavior following direct fluorophore conjugation.

Panel B shows line-profile fluorescence intensity analysis generated from the RAD50-associated confocal dataset. Quantitative fluorescence measurements demonstrated strong focal aggregate enrichment with a maximum fluorescence intensity of 8,648.00 A.U., supporting robust fluorophore conjugation and strong signal generation under confocal acquisition conditions. The fluorescence intensity profile showed multiple discrete RAD50-associated peaks distributed across nuclear regions, consistent with focal DNA-damage response localization and repair complex accumulation.

Panel C presents the Zeiss LSM980 confocal acquisition parameters utilized during image collection. Imaging of the AF488 channel was performed using the 488 nm laser at 1.50% power, with a 1.00 AU pinhole, a scan zoom of 1.6, a pixel time of 0.28 µs, and averaging of 8. These acquisition settings demonstrated strong fluorescence signal generation and preservation of nuclear-associated RAD50 localization under moderate confocal excitation conditions. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) maintained strong fluorescence performance, focal DNA damage-associated localization characteristics, and compatibility with high-resolution confocal fluorescence microscopy workflows following direct fluorophore conjugation.

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N)
Cat ID# - DC 000077

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	46

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	81 Slices (2.40 µm)
Channels	2
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	1085 X 916
Image Size (Scaled)	63.83 µm X 53.88 µm
Bit Depth	16 Bit
Image Center Position	X: 94.12 mm, Y: 52.50 mm
Stage Position	X: 94.12 mm, Y: 52.50 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 51 µm | 1.00 AU / 43 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 505 | Mirror
Laser Wavelength	488 nm @ 1.50% | 405 nm @ 0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6
Rotation	0°
Sampling	1.20
Pixel Time	0.28 µs
Frame Time	5.28 s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	493 | 353
Emission Wavelength	517 | 465
Effective NA	1.4
Detection Wavelength	511 - 550 | 435 - 470
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.1 (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 60.0), Y (Min 0.0 and Max 9080)

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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N)
Cat ID# - DC 000077

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for the identification and processing of DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N), 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 41 of 81, Line Profile Panel B

Z-Stack = 81 Slices (2.40 µm)

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1085 X 916
Image Size (Scaled) = 63.83 µm X 53.88 µm
Bit Depth = 16 Bit
Image Center Position = X: 94.12 mm, Y: 52.50 mm
Stage Position = X: 94.12 mm, Y: 52.50 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 51 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488 nm @ 1.50%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.28 µs
Frame Time = 5.28 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.1 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405 nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.28 µs
Frame Time = 5.28 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 = 435 - 470
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, 2-Dimensional view at Z-plane 41 of 81, shows Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N). A line profile traverses two nuclei to define the region selected for quantitative fluorescence analysis, with the corresponding intensity profile further examined in Panel B.

Profile View Display - Panels B & C

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 60.0), Y (Min 0.0 and Max 9080)

Measured at Z-plane 41 of 81

ZEN Acquisition Info Tab - Panel C

Shown is the acquisition of the 488 channel (RAD50 488 direct conjugate) using the 488 nm laser at 1.50%, demonstrating that image acquisition settings do not artificially produce the signal. The panel also provides full transparency into image acquisition parameters and confocal collection settings used in the dataset.

Summary

Panel A depicts LSM980 confocal fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) following DNA damage induction. Strong nuclear-associated fluorescence localization and pronounced punctate RAD50-associated signal enrichment were observed throughout the nucleus, demonstrating effective target localization and preservation of signal integrity following direct fluorophore conjugation. Distinct focal fluorescence accumulation patterns consistent with DNA damage-associated repair foci formation were observed with minimal background interference, supporting robust fluorophore performance and preservation of biologically coherent localization behavior under confocal fluorescence microscopy conditions. Signal distribution and localization characteristics remained highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, supporting preservation of target specificity and biologically coherent localization behavior following direct fluorophore conjugation.

Panel B shows line-profile fluorescence intensity analysis generated from the RAD50-associated confocal dataset. Quantitative fluorescence measurements demonstrated strong focal aggregate enrichment with a maximum fluorescence intensity of 8,648.00 A.U., supporting robust fluorophore conjugation and strong signal generation under confocal acquisition conditions. The fluorescence intensity profile showed multiple discrete RAD50-associated peaks distributed across nuclear regions, consistent with focal DNA-damage response localization and repair complex accumulation.

Panel C presents the Zeiss LSM980 confocal acquisition parameters utilized during image collection. Imaging of the AF488 channel was performed using the 488 nm laser at 1.50% power, with a 1.00 AU pinhole, a scan zoom of 1.6, a pixel time of 0.28 µs, and averaging of 8. These acquisition settings demonstrated strong fluorescence signal generation and preservation of nuclear-associated RAD50 localization under moderate confocal excitation conditions. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) maintained strong fluorescence performance, focal DNA damage-associated localization characteristics, and compatibility with high-resolution confocal fluorescence microscopy workflows following direct fluorophore conjugation.

Image Correction

NONE

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

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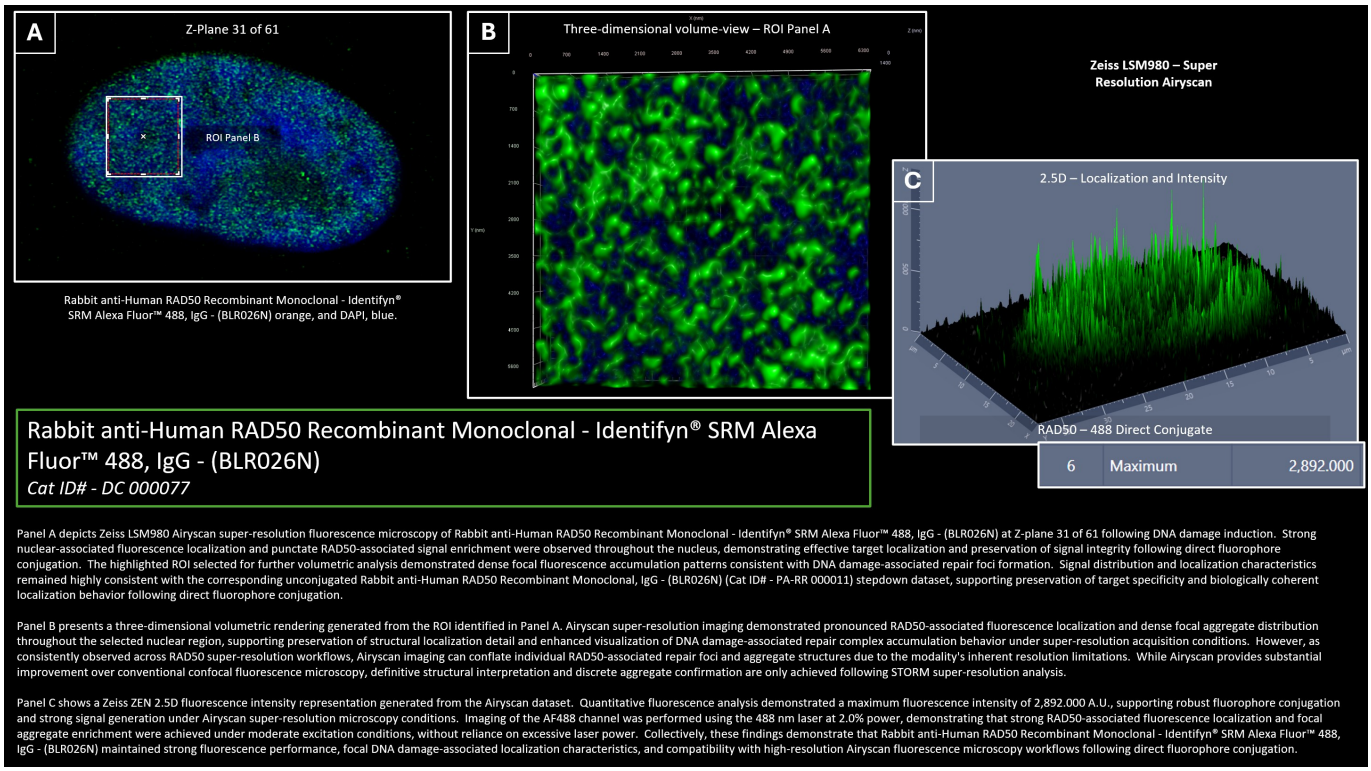
SOURCE PROVENANCE

Catalog	DC-000077
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	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	036C9FBD8C0DEFE79636A455792895D2F660C59CB42280CAF2114F84EF583A05
Method PDF SHA-256	3E5200626CE132D1C55D6470DE8C72A5633469D4138E82807A8A4C8969D2AD1D

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 750
METADATA VALUES	58

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	61 Slices (1.80 µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.03000 µm
Image Size (Pixels)	663 X 1072 | 183 X 171
Image Size (Scaled)	23.40 µm X 37.84 µm | 6.46 µm X 6.04 µm
Bit Depth	16 Bit
Image Center Position	X: 94.81 mm, Y: 52.65 mm
Stage Position	X: 94.81 mm, Y: 52.65 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 250 µm | 5.00 AU / 214 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 505
Laser Wavelength	488 nm @ 2.0% | 405 nm @ 0.2%
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	493 | 353
Emission Wavelength	517 | 465
Effective NA	1.4
Detection Wavelength	499 - 548 | 422 - 477
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	850 V | 750 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution 9.2 (3D, Auto) | SuperResolution 9.4 (3D, Auto)
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)
Render Mode	Surface
Grid distance	5
Angle X	62
Angle Y	-58
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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N)
Cat ID# - DC 000077

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for the identification and processing of DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N), 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 Image at Z-Plane 31 of 61, Panel C, 2.5D Image.

Z-Stack = 61 Slices (1.80 µm)

Channels = 2
Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm
Image Size (Pixels) = 663 X 1072
Image Size (Scaled) = 23.40 µm X 37.84 µm
Bit Depth = 16 Bit
Image Center Position = X: 94.81 mm, Y: 52.65 mm
Stage Position = X: 94.81 mm, Y: 52.65 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, Region of Interest from Panel A, 3-Dimensional Volume View

Z-Stack = 61 Slices (1.80 µm)

Channels = 2
Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm
Image Size (Pixels) = 183 X 171
Image Size (Scaled) = 6.46 µm X 6.04 µm
Bit Depth = 16 Bit
Image Center Position = X: 94.81 mm, Y: 52.65 mm
Stage Position = X: 94.81 mm, Y: 52.65 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488 nm @ 2.0%
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 = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 9.2 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 214 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405 nm @ 0.2%
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 = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 9.4 (3D, Auto)

2-Dimensional View - Panel A

Panel A, 2-Dimensional view at Z-plane 31 of 61, shows Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N). A region of interest (ROI Panel B) was selected from the nucleus to define the area utilized for subsequent three-dimensional volumetric rendering and fluorescence intensity analysis presented in Panels B and C.

3D Image Processing - Panel B

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)

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 = 62
Angle Y = -58
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 RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) direct conjugate Airyscan dataset, demonstrating a peak fluorescence intensity of 2,892.000 A.U., supporting robust fluorophore conjugation and strong signal generation under Airyscan super-resolution fluorescence microscopy acquisition conditions.

Summary

Panel A depicts Zeiss LSM980 Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) at Z-plane 31 of 61 following DNA damage induction. Strong nuclear-associated fluorescence localization and punctate RAD50-associated signal enrichment were observed throughout the nucleus, demonstrating effective target localization and preservation of signal integrity following direct fluorophore conjugation. The highlighted ROI selected for further volumetric analysis demonstrated dense focal fluorescence accumulation patterns consistent with DNA damage-associated repair foci formation. Signal distribution and localization characteristics remained highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, supporting preservation of target specificity and biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents a three-dimensional volumetric rendering generated from the ROI identified in Panel A. Airyscan super-resolution imaging demonstrated pronounced RAD50-associated fluorescence localization and dense focal aggregate distribution throughout the selected nuclear region, supporting preservation of structural localization detail and enhanced visualization of DNA damage-associated repair complex accumulation behavior under super-resolution acquisition conditions. However, as consistently observed across RAD50 super-resolution workflows, Airyscan imaging can conflate individual RAD50-associated repair foci and aggregate structures due to the modality's inherent resolution limitations. While Airyscan provides substantial improvement over conventional confocal fluorescence microscopy, definitive structural interpretation and discrete aggregate confirmation are only achieved following STORM super-resolution analysis.

Panel C shows a Zeiss ZEN 2.5D fluorescence intensity representation generated from the Airyscan dataset. Quantitative fluorescence analysis demonstrated a maximum fluorescence intensity of 2,892.000 A.U., supporting robust fluorophore conjugation and strong signal generation under Airyscan super-resolution microscopy conditions. Imaging of the AF488 channel was performed using the 488 nm laser at 2.0% power, demonstrating that strong RAD50-associated fluorescence localization and focal aggregate enrichment were achieved under moderate excitation conditions, without reliance on excessive laser power. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR026N) maintained strong fluorescence performance, focal DNA damage-associated localization characteristics, and compatibility with high-resolution Airyscan fluorescence microscopy workflows following direct fluorophore conjugation.

Image Correction

NONE

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

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SOURCE PROVENANCE

Catalog	DC-000077
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	58
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
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