

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human RAD50 Polyclonal, IgG

Cat ID# - PA-RP 000007

Identifyn® Secondary Antibodies

Identifyn®SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000015

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 identifying and processing 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.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with [Thermo Fisher Fish Serum Blocker \(cat#37527X3\)](#). The primary antibody, Rabbit anti-Human RAD50 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

The second primary antibody, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962](#).

Microscope

Zeiss LSM 980 Confocal **with NIR Detection**, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Single Plane (2D) - Z-Plane 36 of 77- Panel A

Channels = 3

Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm

Image Size (Pixels) = 1566 X 1566

Image Size (Scaled) = 55.27 µm X 55.27 µm

Bit Depth = 16 Bit

Image Center Position = X: 89.34 mm, Y: 54.32 mm
Stage Position = X: 89.34 mm, Y: 54.32 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Single Plane (2D) - Z-Plane 36 of 77- Panel B, ROI Panel A

Channels = 3
Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm
Image Size (Pixels) = 796 X 783
Image Size (Scaled) = 28.10 μ m X 27.64 μ m
Bit Depth = 16 Bit
Image Center Position = X: 89.34 mm, Y: 54.32 mm
Stage Position = X: 89.34 mm, Y: 54.32 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Panel C, ROI Panel B**Z-Stack = 77 Slices (2.28 μ m)**

Channels = 3
Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm
Image Size (Pixels) = 332 X 317
Image Size (Scaled) = 11.72 μ m X 11.19 μ m
Bit Depth = 16 Bit
Image Center Position = X: 85.47 mm, Y: 48.09 mm
Stage Position = X: 85.47 mm, Y: 48.09 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328 μ m
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 640
Laser Wavelength = **639nm @0.10%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 μ s
Frame Time = 7.82s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan

Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Filter: 9.6 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = **488nm @0.08%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Filter: 8.5 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 214µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **405nm @0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
LSM Scan Speed = 7

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 = Filter: 7.9 (3D, Auto)

2-Dimensional View - Panels A and B

Panel features Rabbit anti-Human RAD50 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) combined with a Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) and DAPI.

Panel B, the 647 channel (53BP1) is changed from the default red to white to enhance visual contrast.

Image was taken from Z-stack Acquisition at plane 36 of 77.

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 3% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel C

Clipping planes are introduced in Panel B to highlight the RAD50 Rabbit Polyclonal within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

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

Position of Clip = -50%
Clip X = 0°
Clip Z = 0°

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

Position of Clip = -8%

Clip Y = 30°

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 = 38%

Clip Y = 30°

Clip Z = 0°

Panel D - STORM Image from the same data set.

Summary -

Gate – Recruitment / Specificity (Go-No-Go)

Using the Identifyn® Standards for Optical Microscopy Methods™, a step-down, multi-modal imaging workflow was applied to evaluate RAD50 recruitment following etoposide-induced DNA damage. HeLa cells were labeled with a rabbit anti-human RAD50 polyclonal IgG, detected with Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibodies, and α -tubulin and DAPI served as cytoplasmic and nuclear references, respectively. Across all imaging views, RAD50 signal was robustly confined to the nuclear compartment, with no detectable cytoplasmic or extranuclear labeling, establishing antibody specificity and passing the go/no-go recruitment gate.

Enrichment – Spatial Context and Signal Validation

Two-dimensional optical sections (Panels A and B) acquired from a 77-slice Z-stack demonstrate a discrete intranuclear RAD50 signal relative to α -tubulin. For visualization, the 647-nm channel was pseudo-colored white instead of its default red, without altering the underlying intensity values. Three-dimensional volume reconstructions (Panel C) reveal punctate and aggregated RAD50 distributions throughout the nuclear volume. Orthogonal clipping and wedge-based projections across X-Y, X-Z, and Y-Z planes confirm spatial continuity and nuclear confinement, enriching the recruitment signal with three-dimensional context.

All data were acquired on a Zeiss LSM 980 with Airyscan detection under low-excitation conditions (639 nm at 0.10%, 488 nm at 0.08%, 405 nm at 0.2%). Quantitative line-profile and histogram analyses show strong signal-to-background ratios and reproducible nuclear intensity distributions, validating signal robustness under conservative acquisition parameters.

Structural Resolution – Deferred Interpretation (Step-Down Progression)

While three-dimensional Airyscan imaging begins to reveal features superficially analogous to those observed by single-molecule localization microscopy, the lateral resolution limit of Airyscan (~120 nm in X-Y) constrains structural interpretation. Apparent RAD50 aggregates at this stage likely represent resolution-limited composites of multiple substructures that are separable only at higher resolution, as demonstrated by STORM imaging of the same dataset (Panel D). Accordingly, although the data confirm

nuclear recruitment and antibody specificity, definitive chromatin-associated structural organization is intentionally deferred to subsequent super-resolution steps.

Aligned Interpretation

Within a CDx framework, confocal and Airyscan imaging establish a binary recruitment gate (presence/absence), provide enrichment through validated three-dimensional localization, and define the transition point at which structural claims require higher-resolution modalities. The Airyscan dataset thus serves as a rigorously controlled intermediate step that preserves assay integrity while enabling progression to SIM, SIM², and STORM for structural stratification of DNA damage-associated RAD50 architecture.

Image Corrections

Identifyfyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast in Panels B and C.

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

