

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG – (BLR026N)***Cat ID# - PA-RR 000011***Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7)***Cat ID# - PA-MM 000002*Identifyn® Secondary Antibody**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000060***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000017*

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 susceptibility to cancer, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 µM etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocking with [Thermo Fisher Fish Serum Blocker \(cat#37527X3\)](#). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG – (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse IgG, F(ab')₂ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI \(cat#P36962\)](#).

Microscope

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

Z Stack - (3D) - Panel A, Z-Plane 68 of 111**Z-Stack = 111 Slices (3.3 µm)**

Channels = 4

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image size (Pixels) = 528 X 673

Image Size (Scaled) = 31.06 μm X 39.59 μm
Bit Depth = 16 Bit
Image Center Position = X: 72.53 mm, Y: 52.26 mm
Stage Position = X: 72.53 mm, Y: 52.26 mm
ROI Center Offset = X: 0.00 μm , Y: 0.00 μm

488 - *Collected but not shown in Confocal Data Analysis (See Multiplex for all Channels)*

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 50 μm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **488 nm @ 0.05%**
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 = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 545
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.4 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 65 μm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = **639 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 = 12

Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.0 (3D, Auto)

555 - *Collected but not shown in Confocal Data Analysis (See Multiplex for all Channels)*

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 56 μ m
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = **561 nm @ 0.4%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.28 μ s
Frame Time = 5.28 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 560 - 600
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.7 (3D, Auto)

DAPI - *Collected but not shown in Confocal Data Analysis (See Multiplex for all Channels)*

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 μ m
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = **405 nm @ 0.3%**

Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.28 μ s
 Frame Time = 5.28 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 435 - 480
 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)

2D View - Panel A

Panel A shows Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG (BLR026N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. The image corresponds to Z-plane 68 of 111, with the line profile indicated and measured 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 35.8), Y (Min 0 and Max 16070)

This panel quantifies RAD50 foci, or resolution-limited aggregates, following etoposide-induced DNA damage. Line profile analysis reveals well-defined intensity peaks and spatial confinement, supporting localized enrichment of RAD50 at damage-associated regions.

Maximum Peak = 15,305.00 A.U.

Zen Acquisition Info Tab - Panel D

Shown are the acquisition parameters for the 647 channel (RAD50), collected using the 639 nm laser at 0.2% power. Scan zoom, scan speed, and line-averaging settings are provided to ensure transparency and reproducibility of line-profile measurements.

Summary

This dataset presents high-resolution confocal images of RAD50 in HeLa cells following etoposide-induced DNA damage, acquired under tightly controlled, reproducible conditions. Using a Zeiss LSM 980 system with 63x/1.40 NA optics, a 3D z-stack (111 slices, 3.3 μm total depth) was collected with consistent acquisition parameters, including low laser power (647 channel at 0.2%), 16-bit depth, and line averaging (8 $\times$), ensuring high signal fidelity with minimal photobleaching or saturation.

RAD50, detected in the 647 channel, exhibits discrete nuclear foci consistent with its recruitment to DNA double-strand breaks as part of the MRN complex. Line profile analysis across selected regions demonstrates well-defined intensity peaks (maximum $\sim 15,305$ A.U.), confirming localized enrichment and spatial confinement of RAD50 within damage-associated regions.

Although additional channels (H2Ax, NPC, DAPI) were acquired as part of a multiplex dataset, the present analysis focuses on RAD50 to provide quantitative clarity and to avoid conflating signal interpretation. Pseudo-coloring of the 647 channel was applied to enhance contrast for measurement without altering underlying signal characteristics.

Biologically, these observations align with established RAD50 function in DNA damage recognition, end tethering, and initiation of repair signaling. The presence of resolution-limited foci reflects accumulation at sites of DNA damage rather than discrete molecular structures, consistent with the limits of confocal imaging and antibody-based labeling.

Overall, this dataset demonstrates a high-quality, reproducible confocal acquisition capturing RAD50 recruitment dynamics, suitable for quantitative analysis and downstream integration into multiplex imaging workflows.

Image Correction

The RAD50 channel (647) was pseudocolored white to enhance visual contrast for measurements.

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

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