

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*

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, 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](#). (Not Shown, but in sample)

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 59 of 161 - Plus DNA Damage, Top

Channels = 3

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

Image Size (Pixels) = 1017 X 1017

Image Size (Scaled) = 59.81 µm X 59.81 µm

Bit Depth = 16 Bit

Image Center Position = X: 92.75 mm, Y: 50.66 mm

Stage Position = X: 92.75 mm, Y: 50.66 mm

ROI Center Offset = X: 0.00 μm , Y: 0.00 μm

Single Plane (2D) - Single Plane- Minus DNA Damage, Bottom

Channels = 1 (647)
Scaling (Per Pixel) = 0.059 μm X 0.059 μm
Image Size (Pixels) = 939 X 758
Image Size (Scaled) = 55.23 μm X 44.58 μm
Bit Depth = 16 Bit
Image Center Position = X: 88.95 mm, Y: 49.33 mm
Stage Position = X: 88.95 mm, Y: 49.33 mm
ROI Center Offset = X: 0.00 μm , Y: 0.00 μm

647 - Etoposide-Induced DNA Damage, Top

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 65 μm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33 μs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 636 - 732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.2 (3D, Auto)

647 - NO Induced DNA Damage, Bottom

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 66 μm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = Mirror
Laser Wavelength = 639nm @0.10%
Laser Blanking = Enabled

Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.30µs
Frame Time = 3.69s
LSM Scan Speed = 12
Scan Direction = Bidirectional

Line Step = 1**Averaging - 8**

Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 635 - 735

Imaging Device = GaAsP-Pmt3**Detector Type = GaAsP-PMT****Detector Gain = 500 V**

Detector Offset = 0

Detector Digital Gain = 1.0

LSM Plus Mode = Filter: 6.6 (3D, Auto)

Profile View Display - Plus DNA Damage, Top

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

Profile View = X and Y axes were set to Auto - **X (Min 0.0 and Max 49.0), Y (Min 0 and Max 21092)**

This panel measures RAD50 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~28K A.U. at its maximum.

Measured at Z-plane 59 of 161.

Profile View Display - Minus DNA Damage, Bottom

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

Profile View = X and Y axes were set to Auto - **X (Min 0.0 and 24.6 Max), Y (Min 163 and Max 7914)**

This panel measures RAD50 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~7K A.U. at its maximum.

Histogram Display - Plus DNA Damage, Top

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

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 2000)

The histogram was generated from the yellow-boxed region shown to quantify the overall RAD50 protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed signal increase relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - *Minus DNA Damage, Bottom*

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

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

The histogram was generated from the yellow-boxed region shown to quantify the overall RAD50 protein within a defined nuclear area in the absence of DNA-damage induction. This measurement provides additional support for the observed signal increase relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusion -

In undamaged cells, RAD50 shows a diffuse nuclear distribution with minimal, low-density aggregation, corresponding to a negative (no-go) recruitment state. Following DNA damage induction, RAD50 redistributes into discrete intranuclear foci or aggregates with an approximately fourfold increase in fluorescence intensity, meeting the predefined positive (go) threshold for damage-associated nuclear recruitment. This transition confirms activation of the DNA damage response and validates RAD50 recruitment as a binary assay readout.

Image Corrections

None

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.