

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG (BLR017M)***Cat ID# - PA-RR 000002*Identifyn® Secondary Antibodies**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, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific***Cat ID# - SA 000012*

Rad51 is a critical protein in the homologous recombination (HR) process, which is a crucial mechanism for repairing DNA double-strand breaks (DSBs) and maintaining genomic stability. Rad51 foci formation in response to DNA damage is a hallmark of the cellular DNA repair process, particularly Homologous Recombination (HR). When cells encounter DNA Double-Strand Breaks (DSBs) or other forms of DNA damage, Rad51 proteins assemble into discrete nuclear foci at the damage sites. Localization and visualization of Rad51 aggregate via immunofluorescent applications allow for the study of Rad51 response kinetics in response to damage, thereby making the study of Rad51 foci formation critical to the study of the cellular response to DNA damage and how it plays a crucial role in the repair process. Rad51 is essential to recognizing DNA damage, activating the Homologous Recombination (HR) pathway, Rad51 filament formation, and DNA repair and resolution...

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with [Thermo Fisher Fish Serum Blocker cat#37527X3](#). The primary antibody, Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour 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 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 127 Slices (3.78µm)

Channels = 3
Scaling (Per Pixel) = 0.030µm X 0.030µm X 0.030µm
Image Size (Pixels) = 1120 X 1120
Image Size (Scaled) = 33.80µm X 33.80µm
Bit Depth = 16 Bit
Image Center Position = X: 78.88mm, Y: 46.61mm
Stage Position = X: 78.88mm, Y: 46.61mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Single Plane (2D) - Panel B and D (Orthogonal and Cut Plane)

Z- Plane 51 of 127

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 57µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @0.7%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.34
Pixel Time = 0.69µs
Frame Time = 8.15s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 656 - 700
Imaging Device = GaAsP-Pmt2
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame

Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.34
Pixel Time = 0.69µs
Frame Time = 8.15s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 478-539
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 183µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.6%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.34
Pixel Time = 0.69µs
Frame Time = 8.15s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410-470
Imaging Device = GaAsP-Pmt1
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% (647), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, 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)

Orthogonal View - Uses Image 51 of the Z-Stack 127 Images - *Panel B*

Cut Lines = X 607, Y 819, Z 51

Line Width = X 10, Y 10, Z 1

Cut Line Opacity = 15

Maximum Intensity Opacity (MIP) and Measure 3D Distance NOT selected

Profile View Display - *Panels C and D*

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

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 2.98), Y (Min 60 and Max 19842)

Summary -

Panel A presents a three-dimensional view of the complete image acquisition, displayed as a maximum projection. Panel B shows an orthogonal view of the same field, excluding the 488 (Tubulin) channel. This panel demonstrates the X-Y image of z-plane 51 out of 127, the X-Z plane (top), and the Y-Z plane (right). The foci of interest are intersected by the green (Y) and red (X) cut lines. While the image may appear to show a "doublet," as we see in super-resolution, diffraction-limited confocal resolution (above 220nm X, Y) cannot resolve this, resulting in RAD51 being displayed as a single foci in the z-planes.

To further demonstrate the resolution of RAD51 foci in confocal imaging, panels C and D present a line profile across the same intersection shown in panel B. The intensity histogram reveals a single peak, indicating no separation and thus representing a single foci. In contrast, the super-resolution techniques—Airyscan, SIM, SIM2, and STORM—provide further structural detail.

Image Corrections -

None

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