

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 = 149 Slices (4.44µm)

Channels = 3
Scaling (Per Pixel) = 33.01nm X 33.01nm X 30.00nm
Image Size (Pixels) = 1000 X 1000
Image Size (Scaled) = 33.01µm X 33.01µm
Bit Depth = 16 Bit
Image Center Position = X: 50.31mm, Y: 47.96mm
Stage Position = X: 50.31mm, Y: 47.96mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel B**Z-Stack = 149 Slices (4.44µm)**

Channels = 3
Scaling (Per Pixel) = 33.01nm X 33.01nm X 30.00nm
Image Size (Pixels) = 151 X 140
Image Size (Scaled) = 4.98µm X 4.62µm
Bit Depth = 16 Bit
Image Center Position = X: 50.31mm, Y: 47.96mm
Stage Position = X: 50.31mm, Y: 47.96mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Single Plane (2D) - Panel D

Z- Plane 50 of 149

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 280µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.14
Pixel Time = 0.52µs
Frame Time = 5.06s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 630 - 700
Imaging Device = Airyscan
Detector Type = GaAsP-PMT

Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.31 AU / 217µm
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 = 2.14
Pixel Time = 0.52µs
Frame Time = 5.06s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 480-550
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 183µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.14
Pixel Time = 0.52µs
Frame Time = 5.06s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8

Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 400-469
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.4 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode "SR"
Super Resolution Auto Filter Selected
Process 3D Current Image
Created

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)

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2% (647), Threshold 2% (DAPI), Ramp 98% (647), Ramp 98% (DAPI), 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)

Cut Plane - Panel A

Clipping Image Process = The Clipping plane is introduced to demonstrate signal specificity to the Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG (BLR017M) to the nuclear compartment following DNA damage.

X-Y = Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment, RAD51 signal, and localization. A red Outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.

Position of Clip = -36%
Clip X = 0°

Clip Z = 0°

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 0.66), Y (Min 0.00 and Max 3067)

Summary -

Panel A presents a three-dimensional view of the complete image acquisition, displayed as a maximum projection. Additionally, Panel A highlights the Region of Interest (ROI) depicted in Panel B. Panel B provides a three-dimensional volume projection of the ROI from Panel A, showcasing RAD51 foci. Within this image, the selected ROI illustrates the "doublet" effect.

Panels C and D utilize line profiles to measure the signal intensity and spatial separation across the "doublet". The super-resolution Airyscan, with a resolution of approximately 120 nm (X, Y), reveals that what appears as a single focus in diffraction-limited images is the "doublet." In contrast, the SIM and SIM2 images from the RAD51 dataset demonstrate a greater separation of this signal, along with elongation or extension of the single focus into a more thread-like structure, observed at the terminal end of the foci. Finally, STORM microscopy can resolve structures that are limited in resolution by the previously mentioned super-resolution methods.

Image Corrections -

None

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Analysis and Presentation by B. T. Bennett

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