

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG - (BLR023N)***Cat ID# - PA-RR 000007*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)***Cat ID# - SA 000063***Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000042*

BRCA1 (Breast Cancer 1) is a protein that plays a crucial role in maintaining the genome's stability and preventing cancer development. BRCA1's primary functions include DNA repair, particularly double-strand breaks that provide function in the homologous recombination pathway.

BRCA1 also significantly regulates the cell cycle, ensuring that cells progress through each phase appropriately. It interacts with various proteins involved in cell cycle checkpoints, helping to prevent cells with damaged DNA from dividing. Additionally, BRCA1 acts as a transcriptional co-regulator, influencing the expression of genes involved in various cellular processes, including DNA repair, cell cycle control, and apoptosis. Finally, BRCA1 is involved in modifying the structure of chromatin (chromatin remodeling); this activity helps regulate gene expression and DNA repair processes.

BRCA1 interacts with numerous proteins, forming complexes involved in the aforementioned cellular processes (DNA repair, cell cycle regulation, transcriptional regulation, and chromatin remodeling). Some fundamental protein-protein interactions of BRCA1 include:

BRCA2: BRCA1 forms a complex with BRCA2, which plays a crucial role in homologous recombination-mediated DNA double-strand break repair.

PALB2 (Partner and Localizer of BRCA2): PALB2 binds to both BRCA1 and BRCA2, facilitating the recruitment of BRCA1-BRCA2 complexes to sites of DNA damage and promoting homologous recombination-mediated repair.

RAD51: BRCA1 interacts with RAD51, an essential protein involved in homologous recombination. This interaction promotes the loading of RAD51 onto single-stranded DNA at sites of DNA damage, facilitating the repair process.

BARD1 (BRCA1-Associated RING Domain protein 1): BARD1 forms a heterodimeric complex with BRCA1, and together they possess E3 ubiquitin ligase activity. This complex plays a role in DNA repair and cell cycle regulation.

CtIP (CtBP-interacting protein): BRCA1 interacts with CtIP, which is involved in the initial processing of DNA double-strand breaks to generate single-stranded DNA ends, a crucial step in homologous recombination-mediated repair.

ATM (Ataxia Telangiectasia Mutated): BRCA1 interacts with ATM, a kinase that plays a central role in the DNA damage response pathway. This interaction is important for activating ATM-mediated signaling in response to DNA damage.

p53: BRCA1 interacts with the tumor suppressor protein p53 and participates in regulating p53-mediated transcriptional responses to DNA damage.

In summary, BRCA1 is essential for maintaining genomic stability and preventing the accumulation of mutations that can lead to cancer development. Mutations in the BRCA1 gene are associated with an increased risk of breast, ovarian, and other cancers, as they impair the protein's ability to carry out its normal functions.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M 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 BRCA1 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, [Mitochondria Rabbit Recombinant Antibody \(MTC02,2860R\)](#), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PPBS. Identifyn™ SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L) 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 Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 54 of 106 total - Panel A

Z Stack - (3D)

Z-Stack = 106 Slices (3.15 μ m)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 106.75 μ m X 106.75 μ m
Bit Depth = 16 Bit
Image Center Position = X: 56.43 mm, Y: 39.32 mm
Stage Position = X: 56.43 mm, Y: 39.32 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Single Plane (2D) - Z-plane 54 of 106 total - Panel B

Z Stack - (3D)

Z-Stack = 106 Slices (3.15 μ m)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 949 X 1299
Image Size (Scaled) = 20.04 μ m X 27.43 μ m
Bit Depth = 16 Bit
Image Center Position = X: 56.43 mm, Y: 39.32 mm
Stage Position = X: 56.43 mm, Y: 39.32 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Panel C**Z-Stack = 106 Slices (3.15 μ m)**

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 420 X 336
Image Size (Scaled) = 8.87 μ m X 7.09 μ m
Bit Depth = 16 Bit
Image Center Position = X: 56.43 mm, Y: 39.32 mm
Stage Position = X: 56.43 mm, Y: 39.32 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 1.13 μ m
Binning Mode = 2,2
Laser Wavelength = 640 nm @3.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 μ m grating

555 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence

Illumination Wavelength = 561
Channel Name = T2-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 0.92 μ m
Binning Mode = 2,2
Laser Wavelength = 561 nm @6.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 μ m grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 μ m
Binning Mode = 2,2
Laser Wavelength = 405 nm @1.50%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 μ m grating

Post Processing - SIM² Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM²
SIM² Settings = Standard
Input SNR = Low
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter

Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 4% all channels, Ramp 96% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 110%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 1°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel displays a 2-dimensional view at z-plane 54 of 106, illustrating the signal and localization of Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (white; the default color, red, has been changed to white in Zeiss Zen software to enhance visual contrast) Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 555 (orange) and DAPI nuclear staining (blue) using super resolution SIM Squared A white box indicates a Region of Interest (ROI) and will be further reduced and investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 54 of 106, helping one visualize the focus we are taking on a specific area of investigation. We present Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG from the ROI in Panel A. BRCA1 is counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (white; the default color, red, has been changed to white in Zeiss Zen software to enhance visual contrast) and DAPI (blue), using super-resolution SIM Squared imaging.

Panel C - This panel showcases the region of interest indicated in Panel B, displaying a three-dimensional volume projection of Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (white; the default color, red, has been changed to white in Zeiss Zen software to enhance visual contrast) Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 555 (orange) and DAPI nuclear staining (blue). We note that ***we made no edits to the image signal acquisition, thresholding, or otherwise;*** however, we did collapse the Z-plane to allow for better visualization, as we are viewing it in 2 dimensions. The view angle was set to 1% and the Scale Z was set to 10%.

Note - It is evident that super-resolution SIM, in this case, SIM Squared, provides a better visualization of BRCA1 spatial localization than super-resolution Airyscan and diffraction-limited techniques. Using **the same sample imaged for SIM**, we demonstrate a gain in resolving the three-dimensional structural localization details of BRCA1 within the nucleus by reducing our axial resolution to approximately 40– 60 nm. Consequently, we must ask the same question with this image further resolved.

Based on thousands of images taken by Identifyn® across various nuclear targets, we face a challenge: either this represents the limit of resolution and may not reflect reality, or it provides a crude visualization of chromatin structure.

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast.

Image by P. Raut

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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