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.

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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 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 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, $F(a'')_2$ (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 PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, $F('b')_2$ (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 µm)

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 µm X 106.75 µm
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 µm, Y: 0.00 µm

Z Stack - (3D) - *Panel B*

Z-Stack = 106 Slices (3.15 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

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Image Size (Pixels) = 1424 X 1542
Image Size (Scaled) = 30.06 µm X 32.56 µm
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 µm, Y: 0.00 µm

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

Z Stack - (3D)

Z-Stack = 106 Slices (3.15 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 490 X 621
Image Size (Scaled) = 10.35 µm X 13.11 µm
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 µm, Y: 0.00 µm

Z Stack - (3D) - *Panel D*

Z-Stack = 45 Slices (1.32 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 145 X 100
Image Size (Scaled) = 3.06 µm X 2.11 µm
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 µm, Y: 0.00 µm

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 µm

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Binning Mode = 2,2
Laser Wavelength = 640 nm @3.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm 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 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @6.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm 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 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @1.50%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

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Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 10.6948 (647), 11.2874 (488), 10.1188 (DAPI)
Fitting = Fast Fit
Normalization = Auto

Split View - *Panel A*

The first panel on the left features Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, $F(a'')_2$ (H + L), the second panel features mitochondria visualized with Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, $F(ab')_2$ (H + L), the third panel features DAPI nuclear staining, and the last panel on the right features the merged image of all channels. This image was taken at Z-Position 54 of 106.

3D Image Processing - *Panel B*

3D Maximum Projection = Precise
Appearance = Threshold 10% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping Enabled
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.

X-Y = Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -36%
Clip X = 0°
Clip Y = 0°

3D Image Processing - *Panel D*

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, 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)

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Summary

Panel A - This panel displays a split view from the image acquisition, at a z-plane 54 out of 106, illustrating the signal and localization of Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG. The left-hand panel highlights the primary antibody, Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The next panel to the right shows a Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 555 (orange). The next panel presents DAPI nuclear staining (blue), while the right panel features a merged image of the three channels. A white box indicates a region of interest (ROI) for further investigation in Panel B.

Panel B - This panel presents a three-dimensional maximum projection of Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG from the ROI in Panel A. It 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 imaging The image features a cut plane on the X-Y axis (blue outline), positioned at -36 degrees, with angles X and Y set to 0 degrees (flat) to enhance the visualization of Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG within the nucleus A white box indicates a region of interest (ROI) for further investigation in Panel C.

Panel C - This panel displays a 2-dimensional image from the ROI in Panel B, at a z-plane 54 out of 106, illustrating the signal and localization of Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (white; the default color, red, has been changed to white in Zeiss Zen software to enhance visual contrast). A red box indicates a region of interest (ROI) for further investigation in Panel D.

Panel D - This panel provides a three-dimensional volume projection of Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG. It 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). No edits to thresholds, no manipulation of any signal, took place; however, the View Angle was reduced to 1% and the Scale Z to 10%, thereby collapsing the Z aberration and providing better 2-dimensional visualization for the viewer.

Note - Using super-resolution Structured Illumination Microscopy (SIM) with the Elyra 5, we observe enhanced structural details of BRCA1, previously seen as "pan-nuclear" or diffuse in diffraction-limited techniques like widefield imaging and confocal microscopy. While super-resolution Airyscan imaging provides more defined images, it still lacks the detail evident with SIM, which achieves 60-80 nm resolution in the X and Y axes.

In Panels A and B, BRCA1 shows some organization, localized to the nucleus and absent in the nucleolus. Panel C highlights a region of interest from Panel B, revealing structural details of BRCA1 at SIM resolution. The organization appears similar to patterns seen with other nuclear proteins.

Panel D features three-dimensional volume projections of the ROI from Panel C to enhance these structures. **Based on thousands of images taken by Identifyn® across various nuclear targets, we face a challenge: either this represents the limit of**

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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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