

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 PBS. 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 Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Aixocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 36 of 70 total - Panel A

Z Stack - (3D)

Z-Stack = 70 Slices (6.9 μ m)

Channels = 3

Scaling (Per Pixel) = 0.143 μ m X 0.143 μ m X 0.100 μ m

Image Size (Pixels) = 1338 X 967

Image Size (Scaled) = 191.14 μ m X 138.14 μ m

Bit Depth = 14 Bit

Image Center Position = X: 60.35 mm, Y: 46.37 mm

Stage Position = X: 60.34 mm, Y: 46.37 mm

ROI Center Offset = X: 1.14 μ m, Y: 0.00 μ m

647 -

Reflector = 50 Cy 5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20.00%
Exposure Time = 200 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.30 μm
Binning Mode = 2,2

555 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20.00%
Exposure Time = 200 ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.10 μm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @8.00%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90 μm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mod" "Optical Sectioning" - enabled correction selected.

Normalization, Phase Corrector, Fourier Filter, and Deconvolution **were NOT used.**

Split View - Panel A

The top-left panel features Rabbit anti-Human BRCA1 Recombinant Monoclonal, Ig, G visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L), the top-right panel features DAPI nuclear staining, the bottom-left panel features mitochondria visualized with Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, F(a'')₂ (H + L), and the bottom-right panel features the merged image of all channels.

Profile View Display - Panel B & C

Z-Position = 36 of 70

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 155.8), Y (Min 1 and Max 1010)

Summary

Panel A - This panel presents a split view that illustrates the signal and localization of Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG at z-plane 36 of the 71 acquired. The upper left section features the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG primary antibody, which is labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right section displays DAPI nuclear staining (blue), while the lower left section shows a Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 555. The lower right presents a merged image combining all three channels.

Panel B - This panel utilizes a line profile display to demonstrate localization and fluorescent intensity. The X-axis represents distance, while the Y-axis indicates intensity. The data reveal that the signals for the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG and DAPI nuclear staining occupy the same location along the X-axis (the nuclear compartment). This finding confirms that the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG and DAPI are colocalized on the Z-plane at 36 of 71, indicating they occupy the same spatial area in the image. The Y-axis also presents the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647, which exhibits comparable fluorescent brightness to DAPI, signifying excellent target signal and overall fluorescence.

Panel C - This panel displays z-plane 36 for both the BRCA1/647 and DAPI channels, with a line drawn across two nuclei to illustrate the source of our measurements in Panel B.

In conclusion, the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG demonstrates nuclear localization with expected minimal cytoplasmic transport, while exhibiting excellent localization and signal intensity.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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