

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.

BRCA 1 also significantly regulates the cell cycle, ensuring 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(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 Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 μ m X 0.110 μ m
Image size (Pixels) = 1173 X 801
Image Size (Scaled) = 128.47 μ m X 87.73 μ m
Bit Depth = 14 Bit
Image Center Position = X: 0.00 μ m, Y: 0.00 μ m
ROI Center Offset = X: 0.00 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 @10.00%
Exposure Time = 120 ms
Excitation Wavelength = 653
Emission Wavelength = 668

Effective NA = 1.4
 Imaging Device = AxioCam 705
 Camera Adapter = 1X
 Depth of Focus = 1.03 μ m
 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 Xyris Lamp Intensity @15.00%
 Exposure Time = 150 ms
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Imaging Device = AxioCam 705
 Camera Adapter = 1X
 Depth of Focus = 0.88 μ m
 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 Xyris Lamp Intensity @8.00%
 Exposure Time = 20 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = AxioCam 705
 Camera Adapter = 1X
 Depth of Focus = 0.72 μ m
 Binning Mode = 2,2

Split View - Panel A

The top-left panel features Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG, 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(ab')₂ (H + L). The bottom-right panel features the merged image of all channels.

Colocalization View - Panel B & C

Horizontal Axis - AF647, Threshold 82, Range Auto

Vertical Axis - DAPI, Threshold 95, Range Auto
Mask = Circle (white)

Summary

Panel A - Split View illustrates the signal and localization of the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG antibody. The upper left section features the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red) The upper right shows DAPI nuclear staining (blue), while the lower left displays a mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 555 (white; the default color, orange, has been changed to white in Zeiss Zen software to enhance visual contrast. The lower right presents a merged image of the three channels.

Panel B - Displays a colocalization view with DAPI (Y-axis) and the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG labeled with Identifyn™SRM Alexa Fluor™ 647 (X-axis). A white circle highlights a Region of Interest (as shown in the right-hand image of Panel B), indicating the area where colocalization was measured. This demonstrates that BRCA1 is strongly colocalized with DAPI, suggesting that, as expected for this protein, it is located within the nuclear compartment.

It is important to note that the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG targets non-modified protein. However, the HeLa cells in this test were exposed to etoposide-induced DNA damage. As indicated in the control image, there is no significant change, suggesting that this antibody does not recognize or display DNA damage-induced "foci," aggregates, or concentrated localization, as it does not recognize modified versions of BRCA1 but the native form.

Image Correction

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L) was pseudo-colored in Zen Software from orange, the default color, to white to provide visual contrast.

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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