

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 engaged 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 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(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 LSM 980 Confocal **with NIR Detection**, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 106 of 221 total - Panel A

Z Stack - (3D) - Panels A & B

Z-Stack = 221 Slices (6.6 µm)

Channels = 3

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image Size (Pixels) = 1121 X 1121

Image Size (Scaled) = 65.93 µm X 65.93 µm

Bit Depth = 16 Bit

Image Center Position = X: 75.28 mm, Y: 54.18 mm

Stage Position = X: 75.28 mm, Y: 54.18 mm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 221 Slices (6.6 µm)

Channels = 3

Scaling (Per Pixel) = $0.059\ \mu\text{m} \times 0.059\ \mu\text{m} \times 0.030\ \mu\text{m}$
Image Size (Pixels) = 934×586
Image Size (Scaled) = $54.93\ \mu\text{m} \times 34.47\ \mu\text{m}$
Bit Depth = 16 Bit
Image Center Position = X: 75.28 mm, Y: 54.18 mm
Stage Position = X: 75.28 mm, Y: 54.18 mm
ROI Center Offset = X: $0.00\ \mu\text{m}$, Y: $0.00\ \mu\text{m}$

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / $64\ \mu\text{m}$
LSM MBS = MBS 488/561/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 639 nm @0.06%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = $0.30\ \mu\text{s}$
Frame Time = 3.69 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645-700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.8 (3D, Auto)

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / $56\ \mu\text{m}$
LSM MBS = MBS 488/561/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 561 nm @0.06%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = $0.30\ \mu\text{s}$

Frame Time = 3.69 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 550-620
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.7 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 µm
LSM MBS = MBS 488/561/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.30 µs
Frame Time = 3.69 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430-490
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.5 (3D, Auto)

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 2% 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)

3D Image Processing - Panel C

3D Maximum Projection = Precise

Appearance = Threshold 2% all channels, Ramp 98% 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 = A clipping plane was introduced to pronounce regions of special interest.

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

Position of Clip = 5%

Clip X = 30°

Clip Z = 0°

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

Position of Clip = 0%

Clip Y = -30°

Clip Z = 0°

Summary

Panel A - This panel displays a 2-dimensional view from z-plane 106 of 221, illustrating the signal and localization of Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG (white, pseudo-colored in Zeiss Zen software from default red for visual contrast), Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 555 (orange), and DAPI nuclear staining (blue). A white box indicates the Region of Interest to be further examined in Panel C.

Panel B – This panel presents a 3-dimensional Maximum Projection of Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG labeled with Identifyn® SRM Alexa Fluor™ 647 (red) and Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 555 (white, pseudo-colored in Zeiss Zen software from default red for visual contrast). DAPI has been omitted to allow better visualization of the BRCA1/647 localization at confocal resolution.

Panel C - This panel presents a 3-dimensional Volume Projection from the ROI in Panel A to demonstrate further the nuclear localization of Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG. The projection shows Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (white, pseudo-colored in Zeiss Zen software from the default red for visual contrast), and

Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 555 (orange), as well as DAPI (blue), at confocal resolution. The image employs a double "wedge" or a double cut plane on the X-Z (green outline) and Y-Z planes (red outline). In the X-Z cut plane, the position is 5%, the angle X is at 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the position is 0%, the angle X is at -30 degrees, and the angle Z is 0 degrees. The wedge allows us from multiple angles to define clearly the BRCA1 localization within the nuclear compartment.

Panel D - A clipped image from Zeiss Zen image acquisition settings for this z-stack comprising 221 planes. **We note that the 639nm laser power is set at 0.06% to obtain maximal imaging of the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG labeled with Identifyn® SRM Alexa Fluor™ 647.**

Panels A, B, and C all demonstrate the Rabbit anti-Human BRCA1 Recombinant Monoclonal IgG labeled with Identifyn® SRM Alexa Fluor™ 647 localized to the nucleus. Additionally, we observe that as we move to confocal resolution, the diffuse pan-nuclear staining seen in the widefield and widefield SIM images now becomes more punctate, providing somewhat better visualization of BRCA1 localization.

Again, it is essential 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 Corrections

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

Panel B - 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.

Panel C - 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 J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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