

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

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 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

Plus Damage – *Panel A*

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 1:25. Cells were washed 3X in P.S. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in PBS and mounted using [ProLong Diamond Antifade Mountant with DAPI \(cat#P36962\)](#).

No Damage – *Panel B*

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with [Thermo Fisher Fish Serum Blocker cat#375 7X3](#). 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® 647 secondary antibody 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, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Z-Stack – (3D) – *Panel A*

Z-Stack = 70 z-planes (6.90 μ m) – Uses Z-plane 36 for Analysis.

Channels = 2
Scaling (Per Pixel) = 0.143 μ m X 0.143 μ m x 100 μ m
Image size (Pixels) = 686 X 409
Image Size (Scaled) = 98.00 μ m X 58.43 μ m
Bit Depth = 14 Bit
Image Center Position = X: 60.35 μ m, Y: 46.37 μ m
Stage Position = X: 60.35 μ m, Y: 46.37 μ m
ROI Center Offset = X: 1.14 μ m, Y: 0.00 μ m

Z-Stack – (3D) – *Panel B*

Z-Stack = 70 z-planes (6.90 μ m) – Uses Z-plane 36 for Analysis.

Channels = 2

Scaling (Per Pixel) = $0.143\mu\text{m} \times 0.143\mu\text{m} \times 100\mu\text{m}$
Image size (Pixels) = 480 X 323
Image Size (Scaled) = $68.57\mu\text{m} \times 46.14\mu\text{m}$
Bit Depth = 14 Bit
Image Center Position = X: $61.99\mu\text{m}$, Y: $46.04\mu\text{m}$
Stage Position = X: $61.99\mu\text{m}$, Y: $46.04\mu\text{m}$
ROI Center Offset = X: $1.14\mu\text{m}$, Y: $0.00\mu\text{m}$

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 = $1.30\mu\text{m}$
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 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 = $0.90\mu\text{m}$
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.**

Profile View Display - Panel A

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 92.7), Y (Min 1 and Max 937)

Profile View Display - Panel B

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 58.2), Y (Min 5 and Max 616)

Summary

Panel A – DNA damage was induced via treatment with 2 μ M Etoposide–spiked complete growth media for 24 hours. This panel presents a profile view of z-plane 36 of 70, showing the optimal signal (center z-position for the nucleus) for the Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red). On the Left is the histogram; the x-axis represents distance, and the y-axis represents fluorescent intensity. ***The Sample Mean (average intensity/distance) is equal to 231.2435 Fluorescent Arbitrary Units (A.U.)*** across the line shown in the right-hand image (407 datapoints), where the measurement was taken.

Panel B – DNA damage was **NOT** induced. This panel presents a profile view of z-plane 36 of 70, showing the optimal signal (center z-position for the nucleus) for the Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red). On the Left is the histogram; the x-axis represents distance, and the y-axis represents fluorescent intensity. ***The Sample Mean (average intensity/distance) is equal to 199.9434889 Arbitrary Units (A.U.)*** across the line shown in the right-hand image (649 data points), where the measurement was taken.

Panel C – This panel displays the ***acquisition settings used for both images***, showing that they are identical, with the same excitation input from the X-Cite Xylis Lamp at 20.00% and exposure times of 200 ms.

Summary - The Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG primary antibody (native or non-modified protein) displays no significant difference in signal localization or protein intensity as a measurement of fluorescence. While the Sample Mean for the etoposide-treated cells is ~14% higher in signal, one could argue that this is attributed to many factors, e.g., sample preparation, cell population, etc. Alternatively, one could say that it represents an increase in BRCA1 protein production in response to the damage, indicating non-modified BRCA1. Both are arguable; however, our data is intended not to infer biological claims, but merely to show localization and comparable fluorescence.

Image Corrections

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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