

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human BRCA2 (FANCD1) Recombinant Monoclonal, IgG – (BLR039P)***Cat ID# - PA-RR 000017***Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7)***Cat ID# - PA-MM 000002*Identifyn® Secondary Antibody**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)***Cat ID# - SA 000063***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000019***Method**

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 µM etoposide-spiked complete growth media for 12 hours, followed by -20 °C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human BRCA2 (FANCD1) Recombinant Monoclonal, IgG – (BLR039P), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X 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. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X 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 µm X 0.110 µm

Image Size (Pixels) = 760 X 735

Image Size (Scaled) = 83.24 µm X 80.50 µm

Bit Depth = 14 Bit

Image Center Position = X: 0.00 mm, Y: 0.00 mm

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

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 @ 30.00%
Exposure Time = 300 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03 μm
Binning Mode = 2,2

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 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 @ 10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72 μm
Binning Mode = 2,2

Split View - panel A

Upper left panel is the Rabbit anti-Human BRCA2 (FANCD1) Recombinant Monoclonal, IgG - (BLR039P) detected with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L). The upper right panel is the Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) detected with Identifyn® SRM Alexa Fluor™ 488 Affinity

Purified Donkey Anti-Mouse, F(ab')₂ (H + L). The lower-left panel is the DAPI nuclear DNA counterstain. The lower-right panel shows the merged view.

Colocalization View - Panel B

Horizontal Axis = AF647, Threshold = 153, Range = Auto

Vertical Axis = AF488, Threshold = 185, Range = Auto

Zeiss Zen Acquisition Settings - Panel C

Shown are the acquisition settings across all three channels, including LED intensity and exposure times, along with other relevant parameters, to ensure methodological transparency.

Summary

Widefield microscopy reveals robust nuclear localization of BRCA2 following 12 hours of treatment with 2 μ M etoposide. In the split-view image, BRCA2 exhibits punctate nuclear organization that remains unresolved at conventional widefield resolution, while the H2Ax and DAPI channels confirm nuclear confinement of the signal. Colocalization analysis demonstrates strong spatial overlap between BRCA2 and H2Ax throughout the nucleus, consistent with BRCA2 recruitment to sites of DNA damage following etoposide treatment. Images were acquired using low-power widefield illumination conditions, with BRCA2 collected at 30% X-Cite Xylis lamp intensity and a 300 ms exposure, H2Ax at 15% lamp intensity and a 150 ms exposure, and DAPI at 10% lamp intensity and a 25 ms exposure. Together, these data establish nuclear localization of BRCA2 following DNA damage induction and provide a baseline reference for subsequent higher-resolution analysis using the IdentifyN® Stepdown™ Methodology.

Image Correction

NONE

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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