Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γH2Ax (Serine 139) Recombinant Monoclonal, Mouse Fc, IgG (BLR014M)
Cat ID# - PA-RR 000005

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, F(ab')$_2$ (H + L)
Cat ID# - SA 000062

Gamma H2AX (γH2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of double-strand breaks (DSBs). When DSBs occur in chromatin, γH2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ-phosphorylation) near the site of the break. This phosphorylation event forms γH2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

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 γH2Ax Recombinant Monoclonal, Mouse Fc, IgG (BLR014M), 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-Mouse, F(a")$_2$ (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, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.143 μm X 0.143 μm
Image size (Pixels) = 919 X 805
Image Size (Scaled) = 131.29 μm X 115.00 μm
Bit Depth = 14 Bit
Image Center Position = X: 62.87 μm, Y: 42.95 μm
Stage Position = X: 62.86 μm, Y: 42.95 μm
ROI Center Offset = X: 1.14 μm, Y: 0.00 μm

647 -
Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655

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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

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 @6.00%
Exposure Time = 12 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

Split View - *Panel A*

The first panel on the left features Rabbit anti-Human γH2Ax Recombinant Monoclonal, Mouse Fc, IgG (BLR014M) visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, $F(a'')_2$ (H + L), the middle panel features DAPI nuclear staining, and the last panel on the right features the merged image of all channels.

2.5D Image Processing

Zeiss Zen Software converts a two-dimensional image into a height map, known as a 2.5D Image. The most significant extension in the Z-direction represents the intensity values, creating a pseudo-three-dimensional image that displays the fluorescent localization or colocalization of the collected signal, along with its intensity in arbitrary units. Here we show the fluorescent intensity of the Human γH2Ax Recombinant Monoclonal, Mouse Fc, IgG2a primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red)

2.5D Display

Render Mode = Surface
Grid distance = 1
Palette and Show Faces at Side are both Selected

2.5D Display Options

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Angle X = 63
Angle Y = -0
Z Scaling = 1.0
Ambient = 50
Specular = 20
Shininess = 50
Light Height = 2.0

Summary

Panel A - Split View illustrates the signal and localization of the Rabbit anti-Human γH2Ax Recombinant Monoclonal, Mouse Fc, IgG2. The left section features the Rabbit anti-Human γH2Ax Recombinant Monoclonal, Mouse Fc, IgG2a primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The middle image shows the DAPI (blue), and the right presents a merged image of the three channels. The merge demonstrates that gamma H2AX Mouse Fc is localized to the nucleus, as indicated by DAPI staining.

Panel B - Zeiss Zen Software converts a two-dimensional image into a height map, known as a 2.5D Image. The most significant extension in the Z-direction represents the intensity values, creating a pseudo-three-dimensional image that displays the fluorescent localization or colocalization of the collected signal, along with its intensity in arbitrary units Here we show the fluorescent intensity of the Human γH2Ax Recombinant Monoclonal, Mouse Fc, IgG2a primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red).

As widefield is the first in our series of the "step down" method, as expected and seen as highly consistent, the staining appears pan-nuclear with some concentrated aggregates commonly referred to as "foci As we move through widefield SIM and Confocal Microscopy, much will remain the same; however, as we enter super-resolution microscopy, we will see that gamma H2AX (Mouse Fc) displays three-dimensional localization and structural details, as we have previously shown in gamma H2AX and its direct conjugates.

Image Correction

None

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

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