

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax (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 (γ H2AX) 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, γ H2AX 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 γ H2AX 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 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 γ H2Ax 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(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 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) - Z-plane 42 of 86 total - Panel A

Z Stack - (3D)

Z-Stack = 86 Slices (8.5 μ m)

Channels = 2

Scaling (Per Pixel) = 0.143 μ m X 0.143 μ m X 0.100 μ m

Image Size (Pixels) = 1283 X 860

Image Size (Scaled) = 183.29 μ m X 122.86 μ m

Bit Depth = 14 Bit

Image Center Position = X: 59.86 mm, Y: 44.39 mm

Stage Position = X: 59.86 mm, Y: 44.39 mm

ROI Center Offset = X: 1.14 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 @15.00%
Exposure Time = 150 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 @10.00%
Exposure Time = 25 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

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.**

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(ab')₂ (H + L), the middle panel features DAPI nuclear staining, and the last panel on the right features the merged image of all channels. This image was taken at Z-Position 42 of 86.

Profile View Display - Panels A & B

Z-Position = 42 of 86
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 151.8), Y (Min 0 and Max 1718)

Summary

Panel A - Split View illustrates the signal and localization of the Rabbit anti-Human γ H2AX Recombinant Monoclonal, Mouse Fc, IgG2a. The left section features the Rabbit anti-Human γ H2AX 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. A line extends across the three nuclei, as this is where we measured the fluorescence spatial and intensity values in panel B.

Panel B - This panel features a line profile display that illustrates localization and fluorescent intensity. The X-axis represents distance, while the Y-axis indicates intensity. The data show that the signals for the Rabbit anti-Human γ H2AX Recombinant Monoclonal antibody (Mouse Fc, IgG2a) labeled with Identifyn® SRM Alexa Fluor™ 647 (red) and DAPI (blue) overlap along the X-axis, which corresponds to the nuclear compartment. This confirms that both the Rabbit anti-Human γ H2AX Recombinant Monoclonal antibody and DAPI are localized on the Z-plane at a position of 42 out of 86, indicating they occupy the same spatial area in the image. Furthermore, the Y-axis displays the Rabbit anti-Human γ H2AX Recombinant Monoclonal antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red), which demonstrates strong fluorescent intensity following Etoposide-induced DNA damage.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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