

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000062

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000022

H2AX, a variant of the histone protein H2A, and its primary function relates to the organization and structure of chromatin, as well as the cellular response to DNA damage. Here are the essential functions and roles of H2AX:

Nucleosome Formation: H2AX, like other histone proteins, contributes to the formation of nucleosomes. A nucleosome is the fundamental unit of chromatin, consisting of DNA wrapped around a core of histone proteins (including two molecules each of H2A, H2B, H3, and H4). This organization plays a crucial role in compacting DNA within the cell nucleus and regulating gene expression by controlling access to DNA.

DNA Damage Response (DDR): One of H2AX's key functions, distinct from those of other histone proteins, is its function in the DNA damage response. When DNA suffers double-strand breaks (DSBs), H2AX is phosphorylated at a specific serine residue (serine 139), leading to the formation of γ-H2AX (gamma H2AX). This phosphorylation serves as an early signal of DNA damage.

Recruitment of DNA Repair Proteins: Phosphorylation of H2AX to form γ-H2AX creates a binding site for various proteins involved in DNA repair. It helps recruit proteins involved in the recognition, signaling, and repair of DNA damage, such as MDC1 (Mediator of DNA Damage Checkpoint 1), 53BP1 (p53-binding protein 1), BRCA1 (Breast Cancer 1), and others. These proteins work together to repair the damage and maintain genomic stability.

Chromatin Remodeling: Through its role in signaling and repair, H2AX plays a role in chromatin remodeling during the DNA damage response. The formation of γ-H2AX marks regions where chromatin structure is altered to allow access for repair proteins and facilitate the repair process.

H2AX's functions are fascinating studies in molecular biology. They involve a complex interplay of structural and signaling roles. Its structural role in chromatin and its specialized role in signaling and facilitating the DNA damage response, particularly in double-strand breaks, are of immense interest. Through its phosphorylation to form γ-H2AX, it acts as a key marker for DNA damage and plays an essential role in the recruitment and organization of proteins implicated in the DNA repair process.

Method

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#37527X3](#). The 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 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.

The second primary antibody, [Actin Recombinant Rabbit Monoclonal Antibody \(JF47-01\)](#), 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™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature 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 Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 44 of 134 total - *Panel A*

Z-Stack = 134 Slices (3.99 µm)

Channels = 3
 Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
 Image Size (Pixels) = 5056 X 5056
 Image Size (Scaled) = 66.72 µm X 66.72 µm
 Bit Depth = 16 Bit
 Image Center Position = X: 63.13 mm, Y: 34.76 mm
 Stage Position = X: 63.13 mm, Y: 34.76 mm
 ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - *Panel B*

Z-Stack = 50 Slices (1.47 µm)

Channels = 3
 Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
 Image Size (Pixels) = 2110 X 2038
 Image Size (Scaled) = 27.84 µm X 26.89 µm
 Bit Depth = 16 Bit
 Image Center Position = X: 63.13 mm, Y: 34.76 mm
 Stage Position = X: 63.13 mm, Y: 34.76 mm
 ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 640
 Channel Name = T1-Axiocam 820-SR
 Excitation Wavelength = 640
 Emission Wavelength = 729

Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 1.13 μm
Binning Mode = 2,2
Laser Wavelength = 640 nm @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 μm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 0.81 μm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.50%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 32 μm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 μm

Binning Mode = 2,2
 Laser Wavelength = 405 nm @6.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM Processing

Processing Dimension = 3D
 Result Sampling = 4
 Process Sampling = N/A
 Channels = 3
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 9.7075 (647), 10.0997 (488), 8.6479 (DAPI)
 Fitting = Fast Fit
 Normalization = Auto

Split View - Panel A

The top-left panel features Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L), the top-right panel shows Actin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels. This split view was taken at Z-Position 44 of 134.

3D Image Processing - Panel B

3D Volume Projection = Precise
 Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A – This panel presents a split-channel view of the 3-dimensional image collected at z-plane 91 of the 181 collected. The upper left-hand panel is the Mouse anti-Human H2Ax Monoclonal, IgG1 counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary (red), the upper right is an Actin primary antibody with Identifyn® SRM Alexa Fluor™ 488 secondary (green). The lower left panel is the DAPI nuclear staining (blue). The bottom right is the merged image of all three channels. We provide this to show several key factors as to nuclear localization: a.) the signal for H2AX is localized to the nucleus, b.) both the DAPI and H2AX are colocalized, and c.) the Actin is not in focus on this plane in the space occupied by the H2AX and DAPI staining, which confirms localization and specificity.

Panel B – This panel presents a 3-dimensional volume projection of Mouse anti-HumanH2Ax Monoclonal, IgG1, counterstained with Identifyn[®] SRM Alexa Fluor[™] 647 secondary(red) and DAPI staining (blue). Panel displays nuclear localization and employing super resolution SIM, the image provides some information as to the distribution of the H2AX, which appears ubiquitous and, like DAPI, at this resolution shows some structural detail not seen in the more common "pan nuclear" diffuse images seen in diffraction-limited microscopy, e.g., widefield, confocal.

Image Corrections

None

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Data Presentation by P.D. Amistoso

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