

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™ 532 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000029***Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000060*

H2AX, a variant of the histone protein H2A, has a primary function that 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™ 532 Affinity Purified Goat Anti-Mouse, F(ab')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™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 5X in 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) = 1216 X 1028
Image Size (Scaled) = 133.18 µm X 112.59 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

532 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 50 ms
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.86 µm
Binning Mode = 2,2

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 = 100 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

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 @15.00%
Exposure Time = 20 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

The top-left panel features Actin visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), the top-right panel shows Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L), the bottom-left panel features DAPI nuclear staining. The bottom-right panel features the merged image of all channels.

Colocalization View - Panel B

Horizontal Axis - DAPI, Threshold 741, Range Auto
Vertical Axis - AF532, Threshold 120, Range Auto

Summary

Panel A - Split View illustrates the signal and localization of Mouse anti-Human H2Ax Monoclonal, IgG1. The Upper left displays Actin primary antibody with Identifyn® SRM Alexa Fluor™ 647, upper right, is Mouse anti-Human H2Ax Monoclonal, IgG1 with an Identifyn® SRM Alexa Fluor™ 532, lower left DAPI nuclear staining (blue), and in the lower right, and the lower left presents a merged image of the three channels.

Panel B - Displays a colocalization view of the DAPI and Mouse anti-Human H2Ax Monoclonal, IgG1, with an Identifyn® SRM Alexa Fluor™ 532 channels, showing that H2AX is within the nuclear compartment as expected for this Histone protein.

Image Correction

None

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Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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