

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**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-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000062*

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*Plus Damage – Panel A*

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, Mouse anti-Human H2Ax Monoclonal, IgG1, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody 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](#).

No Damage – Panel B

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, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody 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) – Panel A

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm
Image size (Pixels) = 766 X 574
Image Size (Scaled) = 109.43 µm X 82.00 µm
Bit Depth = 14 Bit
Image Center Position = X: 81.13 µm, Y: 50.47 µm
Stage Position = X: 81.13 µm, Y: 50.47 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Single Plane (2D) – Panel B

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm
Image size (Pixels) = 789 X 805
Image Size (Scaled) = 112.71 µm X 115.00 µm
Bit Depth = 14 Bit
Image Center Position = X: 49.25 µm, Y: 50.66 µm
Stage Position = X: 49.25 µm, Y: 50.66 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 665
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @8.00%
Exposure Time = 80 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

Summary

Panel A – DNA damage was induced via treatment with 2 μM Etoposide–spiked complete growth media for 24 hours. This panel presents a split-channel view of the 2-dimensional image collected. The left-hand panel is the Mouse anti-Human H2Ax Monoclonal, IgG1 counterstained with Identifyn® SRM AlexaFluor™ 647 secondary (red), and the middle panel is the DAPI nuclear staining (blue). The introduction of DNA damage is conducted to demonstrate that there is no foci or aggregate formation in the presence of DNA damage, thereby separating the non-modified H2AX (native) from variants like gamma H2AX, which aggregate at DNA damage sites in the presence of damage.

Panel B – DNA damage was not induced. This panel presents a split-channel view of the 2-dimensional image collected. The left-hand panel is the Mouse anti-Human H2Ax Monoclonal, IgG1 counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary (red), and the middle panel is the DAPI nuclear staining (blue). The lack of DNA damage demonstrates native localization under non-toxic conditions, which is similar in localization and signal to panel A, where we verify that this is indeed the native, non-modified H2AX mouse monoclonal antibody.

Panel C – This panel displays the acquisition settings used for both images, showing that they are identical, with the same excitation input and exposure times.

Image Corrections

None

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Data Presentation by P.D. Amistoso

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