

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™ 555 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000040

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000076

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™ 555 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™ 680 Affinity Purified Donkey Anti-Rabbit, F(ab')₂ (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 LSM 980 Confocal **with NIR Detection**, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 191 Slices (5.7 µm)

Channels = 3

Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm

Image Size (Pixels) = 1444 X 1444

Image Size (Scaled) = 50.69 µm X 50.69 µm

Bit Depth = 16 Bit

Image Center Position = X: 71.49 mm, Y: 52.70 mm

Stage Position = X: 71.49 mm, Y: 52.70 mm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

555 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 5.00 AU / 286 µm

LMS MBS = MBS 488/561/639, 405/730

LSM SBS = SBS LP 525

Laser Wavelength = 561 nm @0.2%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 2.6

Rotation = 0°

Sampling = 2.00

Pixel Time = 0.71 µs

Frame Time = 7.22 s

LSM Scan Speed = 7

Scan Direction = Bidirectional

Line Step = 1

Averaging = 4

Excitation Wavelength = 553

Emission Wavelength = 568

Effective NA = 1.4

Detection Wavelength = 573-627

Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.4 (3D, Auto)

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 μm
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = SBS SP 640
Laser Wavelength = 639nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.6
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.71 μs
Frame Time = 7.22 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 643-735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.3 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 215 μm
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.6
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.71 μs
Frame Time = 7.22 s

LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 350-499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 7.4 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode "SR"
Super Resolution Auto Filter Selected
Process 3D Current Image
Created

3D Image Processing - Panel A

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)

Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.

X-Z = Active was selected, textured opaque was chosen, and an orange outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -28%
Clip X = -60°
Clip Z = 0°

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% 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)

Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.

X-Z = Active was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 0%

Clip X = 0°

Clip Z = 0°

Summary

Panel A - This panel presents a 3-dimensional Volume Projection of Mouse anti-Human H2Ax Monoclonal, IgG1, counterstained with Identifyn® SRM AlexaFluor™ 555 secondary (orange) and Actin primary antibody with Identifyn® SRM Alexa Fluor™ 680 (red) using super resolution Airyscan. The image employs a cut plane on the X-Z axis (orange box), the angle X has been set at -60 degrees to provide enhanced visualization of the H2AX within the nuclear compartment.

Note that the Mouse anti-Human H2Ax Monoclonal, IgG1, counterstained with Identifyn® SRM Alexa Fluor™ 555 secondary has been pseudo-colored white from its default orange in Zeiss Zen software to enhance visual contrast.

Panel B - This panel presents a 3-dimensional Volume Projection of Mouse anti-Human H2Ax Monoclonal, IgG1, counterstained with Identifyn® SRM AlexaFluor™ 555 secondary (orange) and Actin primary antibody with Identifyn® SRM Alexa Fluor™ 680 (red) using super resolution Airyscan. The image employs a cut plane on the X-Z axis (green line), separating the cut plane, in the forefront of the image is the entire z-stack with both the Actin and H2AX channels, in the back of the image is the X-Z cut plane set at a z-position of 82 of the 191 images collected in the z-stack.

Note that the Actin primary antibody, counterstained with Identifyn® SRM Alexa Fluor™ 680 secondary, has been pseudo-colored dark grey from its default dark red in Zeiss Zen software to enhance visual contrast.

Panel C - Settings for the 555 channel (H2AX/555) image acquisition settings show a 0.2% power on the 561 nm laser.

At Airyscan resolution, the "pan-nuclear" or diffuse nature of H2AX staining becomes structural and may represent chromatin organization, given the ubiquitous nature of H2AX distribution.

Image Corrections

Panel A, the Mouse anti-Human H2Ax Monoclonal, IgG1, counterstained with Identifyn® SRM Alexa Fluor™ 555, was pseudo-colored in Zeiss Zen software from its default color, orange, to white to provide visual contrast between the 555 and 680 channels. In Panel B, the Actin counterstained with Identifyn® SRM Alexa Fluor™ 680 was pseudo-colored

from its default color of dark red to dark grey to provide visual contrast against the 680/555 dye pair.

Image by J.K. Bennett

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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