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 crucial role in chromatin remodeling during the DNA damage response. The formation of γ-H2AX marks regions where chromatin structure is altered, allowing access for repair proteins and facilitating 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

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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 = 136 Slices (4.05 µm)

Channels = 3
Scaling (Per Pixel) = 0.030 µm X 0.030 µm X 0.030 µm
Image Size (Pixels) = 1176 X 1176
Image Size (Scaled) = 35.67 µm X 35.67 µm
Bit Depth = 16 Bit
Image Center Position = X: 95.39 mm, Y: 54.96 mm
Stage Position = X: 95.39 mm, Y: 54.96 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 55 µm
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 561 nm @0.08%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.7
Rotation = 0°
Sampling = 2.33
Pixel Time = 0.29 µs
Frame Time = 1.94 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4

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Detection Wavelength = 550-600
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.8 (3D, Auto)

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 68 µm
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 639 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.7
Rotation = 0°
Sampling = 2.33
Pixel Time = 0.29 µs
Frame Time = 1.94 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 667 - 750
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.4 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 42 µm
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.7
Rotation = 0°
Sampling = 2.33
Pixel Time = 0.29 µs

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Frame Time = 1.94 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410-469
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.8 (3D, Auto)

Split View - *Panel A*

The top-left panel features Actin visualized with Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Rabbit, $F(a'')_2$ (H + L), the top-right panel shows Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) visualized with Identifyn® SRM Alexa Fluor™ 555 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. The view split was taken at Z-Position 69 of 136.

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
ProViewion = 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 = Activate 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 = 30°
Clip Z = 0°

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

Position of Clip = 0%
Clip Y = 30°

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Clip Z = 0°

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™ 680 (red), the upper right is Mouse anti-Human H2Ax Monoclonal, IgG1 with an Identifyn® SRM Alexa Fluor™ 555 (orange), lower left DAPI nuclear staining (light blue), and the lower right presents a merged image of the three channels.

Panel B – This panel presents a 3-dimensional Volume Projection of Mouse anti-Human H2AxMonoclonal, IgG1, counterstained with Identifyn® SRM Alexa Fluor™ 555 secondary (red) and DAPI staining (dark blue) at confocal resolution using LSM Plus (LSMP). The image employs a "wedge" or a double cut plane on the X-Z (green box) and Y-Z planes (red box). The X-Z and Y-Z cut planes, the angles X and Y, respectively, have been set at -30 degrees to provide enhanced visualization of the H2AX within the nuclear compartment.

Image Corrections

None

Image by J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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