

Identifyn Standards for Optical Microscopy Methods™Identifyn Primary Antibody**Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate***Cat ID# - DC 000020*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000017*

Gamma H2AX (γ H2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of DSBs. When DSBs occur in chromatin, γ H2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ -phosphorylation) near the site of the break. This phosphorylation event forms γ H2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M 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, Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody followed this, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody 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](#).

[Control Data - Primary Unconjugated Antibody](#)**Microscope**

Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D)**Z-Stack = 170 Slices (5.07 μ m)**

Channels = 3

Scaling (Per Pixel) = 0.056 μ m X 0.056 μ m X 0.030 μ m

Image Size (Pixels) = 1800 X 1800

Image Size (Scaled) = 101.41 μ m X 101.41 μ m

Bit Depth = 16 Bit
Image Center Position = X: 41.02mm, Y: 45.81mm
Stage Position = X: 41.02mm, Y: 45.81mm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 57μm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.0
Rotation = 0°
Sampling = 1.25
Pixel Time = 0.59μs
Frame Time = 8.91s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 656 - 700
Imaging Device = GaAsP-Pmt2
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 43μm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.0
Rotation = 0°
Sampling = 1.25
Pixel Time = 0.59μs
Frame Time = 8.91s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 493

Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 478 - 539
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 37µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = 0°
 Sampling = 1.25
 Pixel Time = 0.59µs
 Frame Time = 8.91s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 410 - 470
 Imaging Device = GaAsP-Pmt1
 Detector Type = GaAsP-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

3D Image Processing - Panel A

3D Maximum Projection = Precise
 Appearance = Threshold 1% (647), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, 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 - Panel B

The Clipping plane is introduced to demonstrate the specificity of the Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.

X-Y = Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. A red outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.

Position of Clip = 7%

Clip Y = -0°

Clip Z = 0°

Colocalization View - Panel C

Horizontal Axis - 647, Threshold 1356, Range Auto

Vertical Axis - DAPI, Threshold 1711, Range Auto

The

Colocalization View demonstrates that the signal is contained within the nucleus via measurement against the DAPI staining.

Profile View Display - Panel D

Profile Definition = Stroke Thickness 1.75, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 40.6), Y (Min 1076 and Max 51370)

Line Profile view showing the signal contained within the nucleus and the extreme brightness of the gamma H2AX 647 Direct Conjugate.

Image Corrections -

None

Image by E.F. Simon

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