

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™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific***Cat ID# - SA 000034*

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® 555 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 Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D)**Z-Stack = 32 Slices (1.24 μ m)**

Channels = 2

Scaling (Per Pixel) = 13.20nm X 13.20nm X 40.00nm

Image Size (Pixels) = 5056 X 5056

Image Size (Scaled) = 66.72 μ m X 66.72 μ m
Bit Depth = 16 Bit
Image Center Position = X: 51.90mm, Y: 39.91mm
Stage Position = X: 51.90mm, Y: 39.91mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 1.13 μ m
Binning Mode = 2,2
Laser Wavelength = 640nm @3.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 36.5 μ m grating

555 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 583
Effective NA = 1.4
Detection Wavelength = 503 - 546, 576 - 590
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 100ms
Depth of Focus = 0.90 μ m
Binning Mode = 2,2
Laser Wavelength = 561nm @1.0%
Frame Time = 1.33s
Scan Direction = Unidirectional
Grating 32.0 μ m grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 2
Channels = 1
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.6030
Input SNR = N/A
Regularization Weight = N/A
Iterations = N/A
Filter = N/A
Fitting = N/A
Normalization = Automatic
Experimental PSF = N/A

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 4% (647), Threshold 4% (555), Ramp 96% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 116%, Azimuth 71°, Elongation -130°, 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 = The Clipping plane is introduced to demonstrate signal specificity to the nuclear envelope.

X-Y = Active was selected, and Textured Opaque was chosen to remove the obstruction from the image cut plane and highlight the nuclear compartment. A blue outline of the clipped perimeter was shown, and the clip back was selected, while the clip front was NOT selected.

Position of Clip = -19%
Clip X = -0°
Clip Y = 0°

Image Corrections

None

Image by P. Raut

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