

Identifyn Standards for Optical Microscopy Methods™Identifyn Primary Antibody**Rabbit anti-Human γ H2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)***Cat ID# - PA-RR-000004*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Goat Anti-Human, IgG (H + L)***Cat ID# - SA 000069*

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 double-strand breaks (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, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 680 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 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 = 35 Slices (1.7 μ m)**

Channels = 2

Scaling (Per Pixel) = 13.20nm X 13.20nm X 50.00nm

Image Size (Pixels) = 3087 X 3590

Image Size (Scaled) = 40.73 μ m X 47.37 μ m

Bit Depth = 16 Bit

Image Center Position = X: 6.46mm, Y: 255.40mm

Stage Position = X: 6.46mm, Y: 255.40mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m**680 -**

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, 647 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 150ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @9.00%
Frame Time = 1.98s
Scan Direction = Unidirectional
Grating 36.5µm grating

DAPI -

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, 647 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T2-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @6.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm 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 = 9.3886

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 Maximum Projection = Precise
Appearance = Threshold 2% (488), Threshold 4% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 85%, Enabled Tone Mapping
Projection = View Angle 65°, Scale Z 39%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

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Analysis and Presentation by B. T. Bennett

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