

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***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000015*

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.

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™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](#).

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam 705 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3

Scaling (Per Pixel) = 0.110 μ m X 0.110 μ m

Image size (Pixels) = 940 X 696

Image Size (Scaled) = 102.95 μ m X 76.23 μ m

Bit Depth = 12 Bit

Image Center Position = X: 0.00 μ m, Y: 0.00 μ m
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450-490
Filter Emission Wavelength = 500-550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity 20%
Exposure Time = 150ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = AxioCam MR R3
Camera Adapter = 1X
Depth of Focus = 0.80 μ m
Binning Mode = 2,2

680 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625-655
Filter Emission Wavelength = 665-715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25%
Exposure Time = 500ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Imaging Device = AxioCam MR R3
Camera Adapter = 1X
Depth of Focus = 1.09 μ m
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335-383
Filter Emission Wavelength = 420-470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 50ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = AxioCam MR R3
Camera Adapter = 1X
Depth of Focus = 0.72 μ m

Binning Mode = 2,2

Post Processing

None

Image Correction

None

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