

Identifyfyn Standards for Optical Microscopy Methods™Identifyfyn Primary Antibody**Rabbit anti-Human γ H2AX Recombinant Monoclonal - Identifyfyn® SRM Alexa Fluor™ 647 - Direct Conjugate***Cat ID# - DC 000020*

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 explicitly phosphorylated 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 - Identifyfyn® SRM Alexa Fluor™ 647 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI cat#P36962](#).

[Control Data - Primary Unconjugated Antibody](#)

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam MR R3, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.102 μ m X 0.102 μ m

Image size (Pixels) = 417 X 420

Image Size (Scaled) = 42.69 μ m X 43.00 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

647 -

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 @40%

Exposure Time = 200ms

Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam MR R3
Camera Adapter = 1X
Depth of Focus = 1.03µm
Binning Mode = 1,1

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 = 80ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam MR R3
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 1,1

Post Processing**Colocalization View**

Horizontal Axis - 647, Threshold 38, Range Auto
Vertical Axis - DAPI, Threshold 30, Range Auto

Summary

The image and colocalization graphic demonstrate the nuclear localization of γ-H2AX within the nuclear compartment, which results in foci formation following damage, as described in the methods.

Image Correction

None

Image by E.F. Simon

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