

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

Identifyfyn® Secondary Antibodies

Identifyfyn® SRM Alexa Fluor™ 532 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 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 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. Identifyfyn® 532 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 Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.143 μ m X 0.143 μ m

Image Size (Pixels) = 1179 X 1075

Image Size (Scaled) = 168.43 μ m X 153.57 μ m

Bit Depth = 14 Bit

Image Center Position = X: 76.53mm, Y: 52.12mm

Stage Position = X: 76.53mm, Y: 52.12mm

ROI Center Offset = X: 1.14, Y: 0.00

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 @30%

Exposure Time = 150ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.25

Imaging Device = Axiocam 807

Camera Adapter = 1X

Depth of Focus = 1.30µm

Binning Mode = 2,2

532 -

Reflector = AF532-49014

Beam Splitter = 550

Filter Excitation Wavelength = 515 - 545

Filter Emission Wavelength = 555 - 595

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @20%

Exposure Time = 150ms

Excitation Wavelength = 528

Emission Wavelength = 553

Effective NA = 1.25

Imaging Device = Axiocam 807

Camera Adapter = 1X

Depth of Focus = 1.07µm

Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.

Normalization, Phase Correction, Fourier Filter, and Deconvolution were NOT used.

Profile View Display

Profile Definition = Stroke Thickness 2.25, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 101.1), Y (Min 3 and Max 1762)

Summary

Split View with Profile View, created via Zeiss Zen software, demonstrates that the damage-induced gamma-H2AX is localized to the nucleus and is further robust in signal using the X-Cite Xylis Lamp Intensity @30% with a 150ms exposure time for Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate

Image Corrections

None

Image by E.F. Simon

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