

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™ 488 Affinity Purified Donkey Anti-Human IgG (H + L)***Cat ID# - SA 000016*

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® 488 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 LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D)**Z-Stack = 32 Slices (1.55 μ m)**

Channels = 2

Scaling (Per Pixel) = 35.29nm X 35.29nm X 50nm

Image Size (Pixels) = 910 X 766

Image Size (Scaled) = 32.11 μ m X 27.03 μ m

Bit Depth = 16 Bit

Image Center Position = X: 72.12mm, Y: 53.02mm

Stage Position = X: 72.12mm, Y: 53.02mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m**488 -**

Reflector = None

Contrast Method = Fluorescence

Pinhole = 5.00 AU / 219 μ m

LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)

Laser Wavelength = 488nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.59µs
Frame Time = 4.44s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 480-560
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.3 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 181µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @1.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.59µs
Frame Time = 4.44s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 400-460
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 7.8 (3D, Auto)

Post Processing - AiryScan 3D Processing
Display Mode""SR"

Super Resolution Auto Filter Selected

Process 3D Current Image

Created

3D Image Processing

3D Volume Projection = Precise

Appearance = Threshold 4% (488), Threshold 4% (405), Ramp 96% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 139%, Azimuth 56°, Elongation -97°, Enabled Directional Light and Tone Mapping

Projection = View Angle 27°, Scale Z 60%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

DAPI is represented as dark grey instead of its default blue coloring to demonstrate contrast and the specificity and brightness of the Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG primary antibody

Image by P. Raut

Analysis and Presentation by B. T. Bennett

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