

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γH2AX Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate

Cat ID# - DC 000020

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific

Cat ID# - SA 000034

Gamma H2AX (γH2AX) 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, γH2AX 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 γH2AX 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 2μM 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 γH2AX Recombinant Monoclonal - Identifyn® 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. Identifyn® 555 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 LSM 900 Confocal, 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) - Main Image

Z-Stack = 28 Slices (1.35μm) - Subset taken from 56 Slices Selected

Channels = 2

Scaling (Per Pixel) = 48.89nm X 48.49nm X 50.00nm

Image Size (Pixels) = 2064 X 1809

Image Size (Scaled) = 100.92μm X 88.45μm

Bit Depth = 16 Bit
Image Center Position = X: 73.18mm, Y: 51.26mm
Stage Position = X: 73.18mm, Y: 51.26mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Zoomed/Inset**Z-Stack = 56 Slices (2.75µm)**

Channels = 2
Scaling (Per Pixel) = 48.89nm X 48.49nm X 50.00nm
Image Size (Pixels) = 368 X 478
Image Size (Scaled) = 17.99µm X 23.37µm
Bit Depth = 16 Bit
Image Center Position = X: 73.18mm, Y: 51.26mm
Stage Position = X: 73.18mm, Y: 51.26mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 281µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @0.7%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 0.70
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.71µs
Frame Time = 14.66s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 2
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645 - 700
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 9.2 (3D, Auto)

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 246µm

LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 561nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 0.70
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.71µs
Frame Time = 14.66s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 2
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 565 - 609
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.7 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode "SR"
Super Resolution Auto Filter Selected
Process 3D Current Image
Created

3D Image Processing - Main Image

3D Maximum Projection = Precise
Appearance = Threshold 4% (647), Threshold 5% (555), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 95%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 175%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Zoomed Inset

3D Maximum Projection = Precise
Appearance = Threshold 4% (647), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

Main Image - Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was pseudo-colored dark grey within Zeiss Zen software from its default color of orange, providing visual contrast between 555/647. Additionally, the Z-stack was 56 slices in total, and a subset of the slices (28) was created to remove the Alpha Tubulin that was spatially over the nuclear compartment, allowing for better visualization of the gamma H2Ax signal.

Zoomed Inset - No Image corrections were made to the 3D image, and the ***Laser power at 0.7% provided a robust signal for:***

Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate

Image by J.K. Bennett

Copyright 2026 GMD12 LLC.

