

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human NBS1 (P95) Polyclonal, IgG***Cat ID# - PA-RP 000010***Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)***Cat ID# - PA-MM 000001*Identifyn® Secondary Antibody**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000060***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*

NBS1 (Nijmegen breakage syndrome 1) plays a crucial part in the cellular response to DNA double-strand breaks in both the Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ) pathways and is a component of the MRE11/RAD50/NBS1 complex (MRN). NBS1 acts as a sensor of DNA double-strand breaks and, via its interaction with γH2AX, recruits MRE11 and RAD50 to the site of damage, processes the DNA ends, prepares them for repair, and facilitates the recruitment of other repair factors. NBS1 also plays a role in coordinating the cellular response to DNA damage with the cell cycle. It interacts with various cell cycle checkpoint proteins, such as ATM (ataxia-telangiectasia mutated), to halt the cell cycle progression, allowing time for DNA repair. NBS1 also contributes to telomere maintenance and participates in detecting and repairing telomere damage, helping prevent genomic instability associated with telomere dysfunction. Dysfunction or mutations in NBS1 can lead to genomic instability and predispose individuals to cancer and other genetic disorders.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μM etoposide-spiked complete growth media for 24 hours, followed by fixation in -20 °C methanol and blocking with [Thermo Fisher Fish Serum Blocker \(cat#37527X3\)](#). The primary antibody, Rabbit anti-Human NBS1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI \(cat#P36962\)](#).

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 59 of 115, Panel A
Z-Stack = 115 Slices (3.42 µm)

Channels = 2
Scaling (Per Pixel) = 0.042 nm X 0.042 nm X 0.030 nm
Image size (Pixels) = 721 X 827
Image Size (Scaled) = 30.44 µm X 34.92 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.15 mm, Y: 36.14 mm
Stage Position = X: 63.15 mm, Y: 36.14 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - 3D Volume Projection, Panel B
Z-Stack = 115 Slices (3.42 µm)

Channels = 2
Scaling (Per Pixel) = 0.042 nm X 0.042 nm X 0.030 nm
Image size (Pixels) = 199 X 175
Image Size (Scaled) = 8.40 µm X 7.39 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.15 mm, Y: 36.14 mm
Stage Position = X: 63.15 mm, Y: 36.14 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @ 3.0%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 µm grating

555 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Contrast Method = Fluorescence
 Illumination Wavelength = 561
 Channel Name = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 561
 Emission Wavelength = 597
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 120 ms
 Depth of Focus = 0.92 μ m
 Binning Mode = 2,2
 Laser Wavelength = 561 nm @ 2.0%
 Frame Time = 1.33 s
 Scan Direction = Unidirectional
 Grating = 27.5 μ m grating

Post Processing - SIM Processing

Processing Dimension = 3D
 Result Sampling = 4
 Process Sampling = N/A
 Channels = 2
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 9.5745 (647), 10.8128 (555)
 Fitting = Fast Fit
 Normalization = Auto

2-Dimensional View - Panel A

Shows Rabbit anti-Human NBS1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

3D Volume Projection = Precise
 Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Shown from the same product data set to demonstrate the resolution limitations of Lattice SIM, however, better than Airyscan, in determining localization and structural details. Moreover, the signal pattern is consistent with the Airyscan data and is more closely resolution-limited than that of a single-molecule image from STORM.

Summary

Panel A shows a single optical section (Z-plane 59/115) depicting NBS1 and PARP1 distribution following etoposide-induced DNA damage. NBS1 (rabbit polyclonal; ~95 kDa) is detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody (red), and PARP1 (monoclonal) is detected with Identifyn® SRM Alexa Fluor™ 555 goat anti-mouse secondary antibody, pseudocolored white for visual contrast.

Panel B presents a three-dimensional volume reconstruction of the same region. Regions of interest (ROIs), indicated by yellow circles, highlight areas of concentrated signal that show superficial similarity to higher-resolution STORM-resolved structures. Both NBS1 and PARP1 signals are present within these regions, reflecting localized enrichment in DNA damage-associated nuclear compartments; however, the observed structures remain diffraction-limited and do not resolve discrete subunits.

Biologically, PARP1 functions as an early sensor of DNA strand breaks, rapidly binding damaged DNA and initiating PARylation-dependent signaling, while NBS1 (~95 kDa), as part of the MRN complex (MRE11–RAD50–NBS1), is recruited to sites of DNA double-strand breaks and mediates downstream signaling, including ATM activation. Their spatial co-distribution is therefore consistent with coordinated yet mechanistically distinct roles within DNA damage response regions.

Despite improved resolution relative to confocal microscopy, Lattice SIM remains limited (~100 nm lateral resolution) and can conflate closely spaced molecular assemblies into a single apparent structure. As a result, NBS1 and PARP1 signals appear as compact foci or aggregates without resolving the underlying substructure. In contrast, Panel C (STORM) resolves these apparent foci into more complex nanoscale architectures, demonstrating that the structures observed in Panels A and B reflect broader spatial organization rather than true molecular detail.

Image Correction

The PARP1 channel (555) was pseudocolored white to enhance visual contrast between signals.

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.