

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 γ H2AX, 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 μ M 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 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:

Z Stack - (3D) - Panel A**Z-Stack = 81 Slices (8.0 μm)**

Channels = 2

Scaling (Per Pixel) = 0.143 μm X 0.143 μm X 0.100 μm

Image size (Pixels) = 975 X 889

Image Size (Scaled) = 139.29 μm X 127.00 μm

Bit Depth = 14 Bit

Image Center Position = X: 45.29 mm, Y: 49.81 mm

Stage Position = X: 45.29 mm, Y: 49.81 mm

ROI Center Offset = X: 1.14 μm , Y: 0.00 μm

Z Stack - (3D) - Panel B, ROI**Z-Stack = 81 Slices (8.0 μm)**

Channels = 2

Scaling (Per Pixel) = 0.143 μm X 0.143 μm X 0.100 μm

Image size (Pixels) = 310 X 342

Image Size (Scaled) = 44.29 μm X 48.86 μm

Bit Depth = 14 Bit

Image Center Position = X: 45.29 mm, Y: 49.81 mm

Stage Position = X: 45.29 mm, Y: 49.81 mm

ROI Center Offset = X: 1.14 μm , Y: 0.00 μm

647 -

Reflector = 50 Cy 5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715

Contrast Method = Fluorescence

Light Source = X-Cite Xylis Lamp Intensity @ 20.00%

Exposure Time = 200 ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.25

Imaging Device = AxioCam 807 Mono

Camera Adapter = 1x

Depth of Focus = 1.30 μm

Binning Mode = 2,2

555 -

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 @ 20.00%

Exposure Time = 200 ms

Excitation Wavelength = 553

Emission Wavelength = 568

Effective NA = 1.25

Imaging Device = Axiocam 807 Mono
Camera Adapter = 1x
Depth of Focus = 1.00 μm
Binning Mode = 2,2

Post Processing - SIM Processing

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

Normalization, Phase Corrector, Fourier Filter, and Deconvolution **were NOT used.**

3D Image Processing - Panel A

3D Volume Projection = Precise

Appearance = Threshold 13% all channels, Ramp 50% 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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel B

3D Volume Projection = Precise

Appearance = Threshold 13% all channels, Ramp 50% 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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel B

Clipping planes may be introduced in Panel B to highlight the NBS1 Polyclonal and the PARP1 Mouse Monoclonal within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, or, in this case, removing parts of the image in the X-Z and Y-Z planes.

X-Y = Not Used in Data Analysis

X-Z = Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -14%

Clip Y = 30°

Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 0%

Clip Y = -30°

Clip Z = 0°

Summary

Three-dimensional volume-intensity projection (wedge view) with orthogonal plane representation, showing the spatial distribution of NBS1 and PARP1 following etoposide-induced DNA damage. PARP1, an early sensor of DNA strand breaks, and NBS1, a core component of the MRN complex (MRE11–RAD50–NBS1) involved in double-strand break recognition and signaling, exhibit spatial proximity within the imaged volume, consistent with coordinated DNA damage response activity.

Orthogonal reconstructions include the X–Z plane (pseudocolored white) and Y–Z plane (red); the X–Y plane is not shown in this view. These projections provide spatial validation across axes, confirming that signal overlap is not solely due to projection artifacts but reflects true three-dimensional co-distribution within the sampled volume. However, this spatial proximity does not imply direct molecular interaction. PARP1 is rapidly recruited to sites of DNA strand breaks and mediates PARylation-dependent signaling, while NBS1 functions in MRN complex recruitment and downstream DNA damage signaling. Their co-occurrence likely reflects engagement within overlapping or adjacent nuclear microenvironments associated with DNA damage rather than direct binding.

Image Correction

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

Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.