

Identifyfyn Standards for Optical Microscopy Methods™Identifyfyn® 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*Identifyfyn® Secondary Antibody**Identifyfyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000060***Identifyfyn® 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. Identifyfyn® 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. Identifyfyn® 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 \(cat#P36962\)](#).

Single Plane (2D)

Channels = 3

Scaling (Per Pixel) = 0.110 μm X 0.110 μm

Image size (Pixels) = 1216 X 1028
Image Size (Scaled) = 133.18 μm X 112.59 μm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 μm , Y: 0.00 μm
ROI Center Offset = X: 0.00 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 @ 25.00%
Exposure Time = 250 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 1.03 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 @ 15.00%
Exposure Time = 150 ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 0.88 μm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 0.72 μm

Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Rabbit anti-Human NBS1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. The top-right panel shows 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. The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

Colocalization View - Panels B and C

Horizontal Axis - AF647, Threshold 89, Range Auto

Vertical Axis - AF555, Threshold 0.00, Range Auto

Image Measurement = Entire Field

Summary

Panel A – NBS1 (poly) detected with Identifyn® SRM Alexa Fluor™ 647 (red) and PARP1 (mono) detected with Identifyn® SRM Alexa Fluor™ 555 (pseudocolored white), with DAPI marking nuclei (blue). Individual channels and the merged image are shown to illustrate overall signal distribution and nuclear localization across the field.

Panel B – Colocalization analysis of PARP1 (vertical axis) and NBS1 (horizontal axis) following etoposide-induced DNA damage demonstrates strong spatial overlap of signal across the entire acquisition. This distribution is consistent with the coordinated recruitment of both proteins to DNA-damage-associated nuclear regions. PARP1 functions as an early damage sensor and mediator of PARylation-dependent signaling, while NBS1, as part of the MRN complex, participates in double-strand break recognition and repair.

Colocalization at widefield resolution reflects shared spatial distribution within nuclear damage-associated regions and does not imply direct protein–protein interaction.

Image Correction

Identifyn® SRM Alexa Fluor™ 555 was pseudocolored white instead of the default orange for visual contrast.

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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