

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 \(cat#P36962\)](#).

PARP1 Monoclonal counterstained with Identifyfyn® SRM Alexa Fluor™ 555 was acquired but not shown in the data analysis.

Microscope

Zeiss LSM 980 Confocal with **NIR Detection**, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A, Showing Z-Plane 81 of 161
Z-Stack = 161 Slices (4.8 µm)

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 468 X 514
Image Size (Scaled) = 27.53 µm X 30.23 µm
Bit Depth = 16 Bit
Image Center Position = X: 74.44 mm, Y: 45.97 mm
Stage Position = X: 74.44 mm, Y: 45.97 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 64 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = **639 nm @ 0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 µs
Frame Time = 4.97 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.5 (3D, Auto)

555 - Not Shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 56 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = Mirror

Laser Wavelength = **561 nm @ 0.2%**
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.7
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.29 μ s
 Frame Time = 4.97 s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 560 - 620
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 7.9 (3D, Auto)

Profile View Display - Panels A & B

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 29.8), Y (Min 0.0 and Max 39373)

Measured at Z-plane 81 of 161

Zen Acquisition Info Tab - Panel C

Shown are the acquisition parameters for the 647 channel (NBS1) collected using the 639 nm laser at 0.2% power. Scan zoom, scan speed, and line-averaging settings are provided to ensure the transparency and reproducibility of line-profile measurements.

Summary

Panel A – Single z-plane (z = 81 of 161) from a confocal acquisition of NBS1 (rabbit polyclonal primary) detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody. The signal (647) is pseudocolored white to enhance visual contrast. A line profile is overlaid on the image to define the region used for quantitative analysis of NBS1 foci.

Panel B – Corresponding line profile analysis demonstrates a peak fluorescence intensity of 37,854 A.U., indicating substantial local enrichment of NBS1 signal. This level of intensity is consistent with NBS1 aggregation into damage-associated nuclear foci. At confocal resolution, these structures appear as discrete puncta; however, higher-resolution imaging modalities (Lattice SIM and STORM) indicate that such "foci" likely represent clustered or multi-component assemblies rather than singular structures.

Biologically, NBS1 is a critical component of the MRN complex (MRE11–RAD50–NBS1), which is rapidly recruited to sites of DNA double-strand breaks. NBS1 functions in damage recognition, stabilization of the repair complex, and activation of downstream signaling pathways, including ATM-dependent responses. The observed focal accumulation is therefore consistent with the recruitment of the MRN complex to DNA-damage-associated nuclear regions.

Notably, the observed signal intensity was achieved with the 639 nm laser at 0.2% power, supporting the conclusion that the measured fluorescence reflects true protein enrichment rather than signal saturation.

Image Correction

The NBS1 channel (647) was pseudocolored white to enhance visual contrast between signals.

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

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