

Identifyn Standards for Optical Microscopy Methods™Identifyn Primary Antibody**Rabbit anti-Human 53BP1 Polyclonal, IgG***Cat ID# - PA-RP 000008*Identifyn® Secondary Antibodies**Identifyn®SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000015*

53BP1 (P53-binding protein 1) is a crucial player in the DNA damage response (DDR), particularly in the repair of double-strand breaks (DSBs). It has a range of functions that contribute to genomic stability and an appropriate response to DNA damage. The following are the prominent roles and functions of 53BP1 in DNA repair:

DNA Damage Sensing: 53BP1 is among the early proteins recruited to DNA damage sites. It recognizes and binds to the phosphorylated form of H2AX (γ -H2AX), a histone variant phosphorylated at double-strand break sites. This ability to localize to DNA damage sites helps to recruit other proteins involved in DNA repair and signaling.

Non-Homologous End Joining (NHEJ): 53BP1 significantly promotes NHEJ, a pathway to repair double-strand breaks, especially in the G1 phase of the cell cycle when a homologous template is unavailable. 53BP1 promotes this pathway by:

Facilitating Synapsis: 53BP1 helps align the broken DNA ends, preparing them for ligation by the NHEJ machinery.

Restricting End Resection: By inhibiting the end resection process (a step in homologous recombination), 53BP1 ensures that NHEJ remains the primary repair mechanism for DSBs in cells not in the S or G2 phase.

Interaction with Other DNA Repair Proteins: 53BP1 interacts with other proteins to promote NHEJ, such as RIF1 (Rap1 Interacting Factor 1) and the Shieldin complex. These interactions help determine the repair pathway choice, favoring NHEJ over homologous recombination when appropriate.

DNA Damage Signaling: 53BP1 is involved in amplifying DNA damage signaling, contributing to the recruitment of additional repair proteins and activating cell cycle checkpoints. This signaling function helps maintain genomic stability by ensuring that cells do not progress through the cell cycle with unrepaired DNA damage.

Tumor Suppression and Genome Stability: 53BP1 has tumor suppressive properties, given its role in promoting accurate repair and maintaining genomic stability. Its ability to ensure proper repair of DSBs and its interaction with other DDR proteins make it an essential player in preventing mutations and chromosomal aberrations that can lead to cancer.

In summary, 53BP1 is a multifaceted protein in the DNA damage response, promoting non-homologous end joining, facilitating DNA damage signaling, and contributing to genomic stability. Its interactions with other DNA repair proteins and its role in regulating the choice of repair pathways are crucial for maintaining cellular integrity and preventing tumorigenesis.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with [Thermo Fisher Fish Serum Blocker \(cat#37527X3\)](#). The primary antibody, Rabbit anti-Human 53BP1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

The second primary antibody, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) 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](#).

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:

Single Plane (2D) - Z-Plane 72 of 141 - Panel A

Channels = 3

Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm

Image Size (Pixels) = 1793 X 1793

Image Size (Scaled) = 63.29 µm X 63.29 µm

Bit Depth = 16 Bit

Image Center Position = X: 85.47 mm, Y: 48.09 mm

Stage Position = X: 85.47 mm, Y: 48.09 mm

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

Single Plane (2D) - Z-Plane 72 of 141- Panel B, ROI Panel A

Channels = 3

Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm

Image Size (Pixels) = 863 X 640

Image Size (Scaled) = 30.46 µm X 22.59 µm

Bit Depth = 16 Bit

Image Center Position = X: 85.47 mm, Y: 48.09 mm

Stage Position = X: 85.47 mm, Y: 48.09 mm

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

Z Stack - (3D) - Panel C, ROI Panel B

Z-Stack = 141 Slices (4.2 µm)

Channels = 3

Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm

Image Size (Pixels) = 289 X 249

Image Size (Scaled) = 10.20 μm X 8.79 μm
Bit Depth = 16 Bit
Image Center Position = X: 85.47 mm, Y: 48.09 mm
Stage Position = X: 85.47 mm, Y: 48.09 mm
ROI Center Offset = X: 0.00 μm , Y: 0.00 μm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328 μm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 640
Laser Wavelength = **639nm @0.08%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15 μs
Frame Time = 17.86s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Filter: 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250 μm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = **488nm @0.04%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15 μs
Frame Time = 17.86s
LSM Scan Speed = 6

Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Filter: 9.8 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 214µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **405nm @0.3%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15µs
Frame Time = 17.86s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Filter: 8.4 (3D, Auto)

2-Dimensional View - Panels A and B

Panel features Rabbit anti-Human 53BP1 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) combined with a Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) and DAPI,

Panel B, the 647 channel (53BP1) is changed from the default red to white to enhance visual contrast.

Image was taken from Z-stack Acquisition at plane 72 of 141.

3D Image Processing

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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel C

Clipping planes are introduced in Panel B to highlight the 53BP1 Rabbit Polyclonal within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

X-Y = 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 = -49%

Clip X = 0°

Clip Z = 0°

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

Position of Clip = -20%

Clip Y = -30°

Clip Z = 0°

Panel D - STORM Image from the same data set, [DATA](#)

Summary -

Using the Identifyn® Standards for Optical Microscopy Methods™, we continue a step-down, multi-modal imaging analysis to evaluate the localization, organization, and detectability of 53BP1 following etoposide-induced DNA damage. HeLa cells were labeled with a Rabbit anti-human 53BP1 Polyclonal IgG and detected using Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibodies, with α -tubulin and DAPI serving as

cytoskeletal and nuclear references, respectively. Across all imaging modalities and analytical views, the 53BP1 signal was robustly confined to the nuclear compartment, with no detectable cytoplasmic or extranuclear labeling, demonstrating high specificity and low background of the Identifyn® antibody reagents.

Two-dimensional optical sections (Panels A and B) acquired from a 141-slice Z-stack illustrate a discrete intranuclear 53BP1 signal relative to α -tubulin, with enhanced contrast achieved by pseudocoloring the 647-nm channel from its default red to white without altering underlying intensity values. Three-dimensional volume reconstructions (Panel C) generated from the same region of interest reveal punctate and aggregated distributions of 53BP1 throughout the nuclear volume. Orthogonal clipping and wedge-based volume projections further confirm nuclear confinement across X-Y, X-Z, and Y-Z planes, enabling direct visualization of spatial continuity and depth.

All data were acquired on a Zeiss LSM 980 equipped with Airyscan detection under highly sensitive, low-excitation conditions (639 nm at 0.08%, 488 nm at 0.04%, 405 nm at 0.3%), underscoring the robustness of the observed signal while minimizing photobleaching and phototoxicity. Quantitative line-profile and histogram analyses demonstrate strong signal-to-background ratios and reproducible intensity distributions across nuclear regions, supporting consistent detection across the dataset. (confocal)

While three-dimensional Airyscan imaging is beginning to reveal structural features superficially analogous to those observed by single-molecule localization microscopy, the lateral resolution limit of Airyscan (~120 nm in X-Y) constrains structural interpretation. Apparent aggregates at this resolution may represent resolution-limited composites of multiple substructures that are separable only by STORM, as shown in Panel D using the same dataset. Consequently, although these data robustly establish nuclear localization and antibody specificity, they do not permit definitive conclusions regarding chromatin-associated structural organization at this stage of the step-down resolution workflow.

Given the central role of 53BP1 in DNA double-strand break signaling, pathway choice, and genome stability, accurate identification of its nuclear localization and distribution following etoposide-induced damage is essential for interpreting repair pathway engagement. The Super Resolution Airyscan data continues to establish a rigorous, low-power imaging foundation upon which higher-resolution modalities can be applied to resolve damage-induced chromatin architecture with greater structural fidelity.

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast in Panels B and C.

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

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