

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 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 = 57 Slices (5.6 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 1307 X 1045
Image Size (Scaled) = 186.71 µm X 148.29 µm
Bit Depth = 14 Bit
Image Center Position = X: 63.02 µm, Y: 50.49 µm
Stage Position = X: 63.02 µm, Y: 50.49 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Single Plane (2D) - Panel B, Z-Plane 29 of 57, (Dimension Shown in Panel A)

Z Stack - (3D) - Panel C

Z-Stack = 57 Slices (5.6 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 682 X 695
Image Size (Scaled) = 98.86 µm X 99.29 µm
Image Center Position = X: 63.02 µm, Y: 50.49 µm
Stage Position = X: 63.02 µm, Y: 50.49 µm
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 @15.00%
Exposure Time = 150 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

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 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 = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.72 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 Maximum Projection = Precise

Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, 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 C

3D Maximum Projection = Precise

Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, 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 green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 0%

Clip X = 0°

Clip Z = 0°

X-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 = -17%

Clip Y = 45°

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 = -16%

Clip Y = -45°

Clip Z = 0°

Summary

Widefield structured illumination microscopy (Apotome) imaging of 53BP1 following etoposide-induced DNA damage reveals robust and highly specific nuclear localization. Panel A presents the full three-dimensional acquisition, rendered as a maximum-intensity projection, that captures the global nuclear distribution of 53BP1 across the entire z-stack. Panel B highlights a single optical section (Z-plane 29 of 57), with a defined region of interest selected for higher-order three-dimensional analysis. This region is further examined using a three-dimensional maximum-intensity projection (wedge view), which illustrates orthogonal planes (X-Y, green; X-Z, red; Y-Z, blue) and enables visualization of 53BP1 localization across all spatial axes. The signal is confined to the nuclear compartment, with no detectable cytoplasmic or nucleolar staining, consistent with 53BP1's established role as a chromatin-associated DNA damage response factor. The clarity, confinement, and reproducibility of the nuclear signal underscore the high specificity and low background of the polyclonal 53BP1 antibody, supporting its suitability for three-dimensional imaging of the DNA damage response under low-phototoxicity conditions.

Image Correction

Identifyfyn® 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 Panel C.

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

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