

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 30 of 64 - Panels A & E

Z Stack - (3D) - Panel B

Z-Stack = 64 Slices (1.89 µm)

Channels = 3

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image Size (Pixels) = 1017 X 1017

Image Size (Scaled) = 59.81 µm X 59.81 µm

Bit Depth = 16 Bit

Image Center Position = X: 86.79 mm, Y: 50.23 mm

Stage Position = X: 86.79 mm, Y: 50.23 mm

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

647 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00AU / 65µm

LMS MBS = MBS 488/639 & 405/730

LMS SBS = SBS SP 615

Laser Wavelength = **639nm @0.1%**

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 2.2

Rotation = 0°

Sampling = 1.20

Pixel Time = 0.33µs

Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 732
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)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **488nm @0.04%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 43µm
LMS MBS = MBS 488/639 & 405/730

LMS SBS = Mirror
 Laser Wavelength = **405nm @0.3%**
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.2
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.33µs
 Frame Time = 3.36s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 430 - 480
 Imaging Device = GaAsp-Pmt3
 Detector Type = GaAsp-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.5 (3D, Auto)

2-Dimensional View - Panel A

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).

Image was taken from Z-stack Acquisition at plane 30 of 64

3D Image Processing - Panel B

3D Maximum Projection = Precise
 Appearance = Threshold 2% 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)

Note that the 53BP1, counterstained with 647, was pseudo-colored white from red in Zeiss Zen, to enhance visual contrast.

LSM 980 Zen Acquisition Info Tab - Panel C

Shown is the acquisition of the 647 channel (53BP1 Rabbit Polyclonal with Identifyn 647 secondary) using the **639nm laser at 0.1%**.

Not shown, the 488 channel (Alpha Tubulin) using the **488nm laser at 0.04%**, and DAPI using the **405nm laser at 0.3%**

Profile View Display - Panels D & E

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 37.6), Y (Min 0 and Max 28610)

This panel measures 53BP1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately 28K A.U. at its maximum.

Measured at Z-plane 30 of 64

Histogram Display - Panels E & F

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 20000)

The histogram was generated from the yellow-boxed region shown in Panel E to quantify the overall 53BP1 protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed signal increase relative to the control condition (see control), complementing the line-profile analysis presented alongside it.

Summary -

Confocal imaging was used to characterize the spatial distribution and intensity of 53BP1 following DNA damage, combining two-dimensional visualization with three-dimensional reconstruction and quantitative signal analysis.

Panel A presents a representative two-dimensional optical section (Z-plane 30 of 64) acquired from a Z-stack, showing Rabbit anti-Human 53BP1 Polyclonal IgG detected with an Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibody, together with α -tubulin labeled using a mouse monoclonal primary and an Identifyn® SRM Alexa Fluor™ 488 secondary antibody. This view demonstrates strong nuclear enrichment of 53BP1 relative to the cytoskeletal counterstain, consistent with a DNA damage-associated chromatin response.

Panel B displays three-dimensional image processing of the same acquisition rendered as a precise maximum-intensity projection, using standardized thresholding and tone-mapping parameters to preserve structural fidelity and quantitative integrity. The projection highlights discrete intranuclear 53BP1 foci and aggregates distributed throughout the nuclear volume, with minimal background and no detectable cytoplasmic signal. For visual clarity, the 647 nm channel corresponding to 53BP1 was pseudocolored white instead of its default red, without altering the underlying intensity values.

Panel C documents the acquisition conditions for the 53BP1 channel, demonstrating that a high-contrast nuclear signal was achieved using extremely low laser power (639 nm at 0.1%), with similarly low excitation applied for the 488 nm α -tubulin and 405 nm DAPI channels (not shown). These conditions underscore the sensitivity of the detection and minimize photobleaching and phototoxicity.

Quantitative analysis is shown in **Panels D and E**, where line-profile measurements across an arbitrary intranuclear axis reveal prominent 53BP1 DNA damage-induced foci, with peak signal intensities reaching approximately 28,000 arbitrary units at Z-plane 30 of 64. Complementary histogram analysis (**Panels E and F**), generated from a defined nuclear region of interest, quantifies the overall 53BP1 response following damage induction and demonstrates a marked shift in signal distribution relative to control conditions. Together, the spatial confinement, high signal-to-background ratio, and reproducibility across orthogonal analyses support the specificity and performance of the polyclonal 53BP1 antibody for quantitative three-dimensional DNA damage response imaging under low-excitation conditions.

Image Corrections

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 Panels B and E.

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

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