

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

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 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 sites of DNA damage. 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 to prepare 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](#). (Not Shown, but in sample)

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 - Plus DNA Damage, Top

Channels = 1 (647)
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

Single Plane (2D) - Single Plane - Minus DNA Damage, Bottom

Channels = 1 (647)
Scaling (Per Pixel) = 0.059 µm X 0.059 µm
Image Size (Pixels) = 1737 X 1737
Image Size (Scaled) = 102.19 µm X 102.19 µm
Bit Depth = 16 Bit
Image Center Position = X: 82.25 mm, Y: 48.06 mm
Stage Position = X: 82.25 mm, Y: 48.06 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 - Etoposide-Induced DNA Damage, Top

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 65µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @0.10%
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)

647 - NO Induced DNA Damage, Bottom

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 66µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = Mirror

Laser Wavelength = 639nm @0.10%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 1.3

Rotation = 0°

Sampling = 1.20

Pixel Time = 0.22µs

Frame Time = 6.49s

LSM Scan Speed = 11

Scan Direction = Bidirectional

Line Step = 1

Averaging - 8

Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 668

Imaging Device = GaAsP-Pmt3

Detector Type = GaAsP-PMT

Detector Gain = 500 V

Detector Offset = 0

Detector Digital Gain = 1.0

LSM Plus Mode = Filter: 6.6 (3D, Auto)

Profile View Display - Plus DNA Damage, Top

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 ~44K A.U. at its maximum.

Measured at Z-plane 71 of 141.

Profile View Display - *Minus DNA Damage, Bottom*

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 28.3 Max), Y (Min 36 and Max 7432)**

This panel measures 53BP1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~6K A.U. at its maximum.

Histogram Display - *Plus DNA Damage, Top*

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 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, complementing the line-profile analysis presented alongside it.

Histogram Display - *Minus DNA Damage, Bottom*

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 5000)

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

Control Conclusion -

In undamaged cells, 53BP1 displays a predominantly diffuse nuclear distribution with only weak, low-density aggregate formation. Following induction of DNA damage, 53BP1 redistributes into discrete intranuclear foci or aggregates, accompanied by an

approximately threefold increase in fluorescence signal intensity. This shift is consistent with enhanced nuclear accumulation and damage-associated recruitment of 53BP1, reflecting activation of the DNA damage response.

Image Corrections

None

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

