

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human BAP1 Polyclonal***Cat ID# - PA-RP 000012*Identifyn® Secondary Antibody**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057*

BAP1 (BRCA1-associated protein 1) is a deubiquitinase with critical roles in DNA repair, chromatin dynamics, and cell cycle regulation. Its ability to interact with other proteins central to the DNA damage response is significant in maintaining genomic stability. BAP1 has several identified protein-protein interactions within the HR pathway, which are critical to DNA Damage Response (DDR) signaling.

BAP1 and BRCA1, BARD1, Rad51 and Others

BRCA1 is a tumor suppressor gene critical for homologous recombination (HR), a high-fidelity DNA repair pathway for double-strand breaks. BAP1's interaction with BRCA1 and other DNA repair proteins, such as BARD1 and Rad51, is linked to its deubiquitinating activity, suggesting that it regulates critical protein function during DNA repair. This relationship may influence BRCA1's stability, recruitment to DNA damage sites, or ability to mediate HR. This interaction underlines the importance of BAP1 in promoting accurate DNA repair and maintaining genomic stability.

BAP1 and ATM

ATM (ataxia-telangiectasia mutated) is a central kinase in the DNA damage response, primarily activated by DNA double-strand breaks. It phosphorylates various downstream targets to initiate DNA repair pathways, including homologous recombination and non-homologous end joining (NHEJ). While the direct interaction between BAP1 and ATM isn't as well-documented as other interactions, BAP1 may indirectly influence ATM's activity by affecting the stability or function of proteins involved in DDR. Deubiquitination (BAP1) and phosphorylation (ATM) may well affect how cells respond to DNA damage.

BAP1 and HERC2

HERC2 is an E3 ubiquitin ligase involved in regulating protein ubiquitination. It has a known role in genomic stability and is implicated in the DNA damage response. BAP1's interaction with HERC2 likely involves deubiquitination, suggesting that BAP1 could regulate HERC2's activity or modulate its effects on other proteins in the DNA repair pathways. This interaction could be critical in balancing ubiquitination and deubiquitination, impacting DNA repair processes and cell cycle control.

ASXL1/ASXL2

ASXL1 and ASXL2 (additional sex combs-like 1 and 2) are other key interactors of BAP1. This complex can influence chromatin remodeling, potentially affecting the accessibility of DNA repair proteins to sites of DNA damage.

Implications in DNA Repair

These interactions underscore BAP1's involvement in DNA repair pathways and the broader DNA damage response. By interacting with BRCA1, BAP1 may facilitate homologous recombination, while interactions with ATM and HERC2 highlight its role in broader DDR signaling. Critical interaction with ASXL1/ASXL2 highlights its importance in chromatin remodeling, potentially affecting the accessibility of DNA repair proteins to sites of DNA damage. BAP1's deubiquitinating activity can stabilize essential proteins, promote the proper assembly of repair complexes, and maintain chromatin structure, all of which are necessary for efficient DNA repair and genomic integrity. Mutations in BAP1 that disrupt these interactions can increase genomic instability and are associated with various cancers, underscoring the clinical relevance of these protein interactions for understanding disease mechanisms and developing therapeutic strategies.

Method

Plus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocked with [Thermo Fisher Fish Serum Blocker cat#37527X3](#). The primary antibody, Rabbit anti-Human BAP1 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™ 594 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 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI \(cat# P36962\)](#).

Minus Damage -

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 BAP1 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™ 594 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 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:

Z Stack - (3D) - 2D at Z-Plane 70 of 141, Plus Etoposide.
Z-Stack = 141 Slices (4.20 μ m)

Channels = 1
Scaling (Per Pixel) = 0.059 μ m X 0.059 μ m X 0.030 μ m
Image size (Pixels) = 503 X 684
Image Size (Scaled) = 29.59 μ m X 40.23 μ m
Bit Depth = 16 Bit
Image Center Position = X: 93.57 mm, Y: 45.85 mm
Stage Position = X: 93.57 mm, Y: 45.85 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Single Plane (2D) - 2D at Z-Plane 76 of 151, No Etoposide.

Channels = 1
Scaling (Per Pixel) = 0.059 μm X 0.059 μm
Image size (Pixels) = 1168 X 949
Image Size (Scaled) = 68.71 μm X 55.83 μm
Bit Depth = 16 Bit
Image Center Position = X: 91.93 mm, Y: 51.77 mm
Stage Position = X: 91.93 μm , Y: 51.77 μm
ROI Center Offset = X: 0.00 mm, Y: 0.00 mm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 59 μm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = **561 nm @ 0.5%**
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 = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590 - 645
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.6 (3D, Auto)

594 - Minus Damage - noting the same laser input power and conditions

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 59 μm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = **561 nm @ 0.5%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9

Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 μ s
Frame Time = 3.89 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590 - 645
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.0 (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 34.9), Y (Min 0.0 and Max 26104)**

This panel measures BAP1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~26K A.U. at its maximum.

Measured at Z-plane 70 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 Max 58.3), Y (Min 0.0 and Max 8.19)**

This panel measures BAP1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~7K A.U. at its maximum.

2-Dimensional Image

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 0.0 and Max 200000)

Arithmetic Mean intensity = 1,752.556

The histogram was generated from the yellow-boxed region shown to quantify the overall BAP1 protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed increase in signal 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 0.0 and Max 1000000)

Arithmetic Mean intensity = 616.777

The histogram was generated from the yellow-boxed region shown to quantify the overall BAP1 protein within a defined nuclear area in the absence of DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusion -

In undamaged cells, BAP1 exhibits a predominantly diffuse nuclear distribution when detected using the Identifyn® Goat anti-Rabbit 594 secondary antibody. Following etoposide-induced DNA damage, BAP1 shows increased nuclear intensity and reorganizes into distinct focal aggregates, consistent with its recruitment to DNA damage-associated repair regions. Quantitatively, etoposide treatment resulted in an approximately 3-fold increase in arithmetic mean fluorescence intensity and an approximately 2-fold increase in maximum fluorescence A.U., supporting damage-induced enrichment and localized concentration of BAP1 within the nucleus.

This redistribution is consistent with the established role of BAP1 as a chromatin-associated tumor suppressor involved in DNA double-strand break repair and chromatin remodeling during the DNA damage response. The observed focal accumulation reflects recruitment into damage-responsive nuclear microenvironments rather than diffuse basal chromatin association observed under untreated conditions.

At diffraction-limited confocal resolution, these apparent puncta likely represent clustered or conflated repair-associated assemblies rather than singular molecular structures, and spatial proximity to other repair proteins does not imply direct molecular interaction but coordinated engagement within DNA damage response pathways. Notably, identical acquisition settings were maintained across conditions, with the 561 nm laser operated at only 0.5% power during confocal imaging, supporting the conclusion that the observed fluorescence enrichment reflects biologically relevant recruitment rather than overexposure or detector saturation artifacts.

Image Correction

NONE

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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