

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human BAP1 Polyclonal, IgG***Cat ID# - PA-RP 000012***Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)***Cat ID# - PA-MM 000002*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000046***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000015*

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

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, DNA damage was induced by treatment with 2 μ M etoposide-spiked complete growth media for 24 hours, 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 3 times in 1X 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 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X 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 5 times in 1X 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) - Panel A, Z-Plane 70 of 141 is Shown.
Z-Stack = 141 Slices (4.2 μ m)

Channels = 3
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

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 59 μ m
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 μ s
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)

488 - Acquired but not shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51 μ m
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **488 nm @ 0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 μ s
Frame Time = 4.97 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8

Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 549
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.9 (3D, Auto)

DAPI - Acquired but not shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 43 μ m
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = **405 nm @ 0.3%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 μ s
Frame Time = 4.97 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430 - 490
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)

Profile View Display - Panels A & B

Profile Definition = Stroke Thickness 1, 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)

Measured at Z-Plane 70 of 141

Four discrete BAP1-enriched nuclear foci with measured peak fluorescence values of 24,861 A.U., 21,935 A.U., 18,441 A.U., and 14,462 A.U. respectively.

Zen Acquisition Info Tab - Panel C

Shown are the acquisition parameters for the 594 channel (BAP1) collected using the 561 nm laser at 0.5% power. Scan zoom, scan speed, and line-averaging settings are provided to ensure the transparency and reproducibility of line-profile measurements.

Summary

Panel A – Single z-plane ($z = 70$ of 141) from a Zeiss LSM980 confocal acquisition of Rabbit anti-Human BAP1 Polyclonal, IgG detected with the Identifyn® Goat anti-Rabbit 594 secondary antibody. The 594 signal is pseudo-colored white to enhance visualization of nuclear signal architecture and damage-associated enrichment. A line profile overlay defines the region used for quantitative fluorescence analysis of BAP1-positive nuclear foci following etoposide-induced DNA damage.

Panel B – Corresponding fluorescence intensity analysis identifies four discrete BAP1-enriched nuclear foci with measured peak fluorescence values of 24,861 A.U., 21,935 A.U., 18,441 A.U., and 14,462 A.U., respectively. These localized increases in fluorescence intensity are consistent with recruitment and concentration of BAP1 within DNA damage-associated nuclear repair domains. At diffraction-limited confocal resolution, these structures appear as discrete punctate foci; however, comparison with higher-resolution imaging modalities, including Airyscan, Lattice SIM, and STORM, suggests that these puncta likely represent conflated multi-protein repair microenvironments rather than singular molecular assemblies.

Panel C – Confocal laser acquisition settings exported directly from Zeiss ZEN software demonstrating that the 561 nm laser input was operated at 0.5% power during image acquisition, supporting that the measured fluorescence enrichment reflects a biologically relevant signal rather than detector saturation or overexposure artifacts.

Biologically, BAP1 is a chromatin-associated tumor suppressor involved in DNA double-strand break repair and chromatin remodeling following genotoxic stress. The observed focal enrichment is consistent with recruitment of BAP1 into H2AX-associated damage-responsive nuclear regions following etoposide-induced DNA damage.

Image Correction

The BAP1 channel (594) was pseudo-colored white to enhance visual contrast between signals.

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

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