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

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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 µM 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) - *2D at Z-Plane 84 of 171, Panel A*
Z-Stack = 171 Slices (5.1 µm)

Channels = 2
Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm
Image size (Pixels) = 1004 X 924
Image Size (Scaled) = 35.44 µm X 32.61 µm
Bit Depth = 16 Bit
Image Center Position = X: 93.76 mm, Y: 45.62 mm

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Stage Position = X: 93.76 mm, Y: 45.62 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - *3D Volume Projection, Panel B*
Z-Stack = 171 Slices (5.1 µm)

Channels = 2
Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm
Image size (Pixels) = 193 X 209
Image Size (Scaled) = 6.81 µm X 7.38 µm
Bit Depth = 16 Bit
Image Center Position = X: 93.76 mm, Y: 45.62 mm
Stage Position = X: 93.76 mm, Y: 45.62 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = **639 nm @ 0.3%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 10.0 (3D, Auto)

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 286 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS LP 525

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Laser Wavelength = **561 nm @ 1.20%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10 µs
Frame Time = 18.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 573 - 627
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **488 nm @ 0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10 µs
Frame Time = 18.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 9.4 (3D, Auto)

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DAPI - *Not Shown in Data Analysis, but Collected in Image Acquisition*
Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 235 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **405 nm @ 0.4%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10 µs
Frame Time = 18.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 350 - 499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.0 (3D, Auto)

2-Dimensional View- *Panel A*

Shows Rabbit anti-Human BAP1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Rabbit, IgG (H + L) and Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 affinity-purified Donkey anti-Mouse, IgG (H + L). Image is at z-plane 84 of 171

3D Image Processing - *Panel B*

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - *Panel B*

Clipping planes are introduced in Panel B to highlight the Rabbit anti-Human BAP1 Polyclonal, IgG, and the Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) within

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the nuclear compartment by adjusting or cutting away portions of the image in the X-Y, X-Z, and Y-Z planes.

X-Y = Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip = -5%
Clip Y = 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 = 3%
Clip Y = -30°
Clip Z = 0°

STORM- *Panel C*

Shown from the same product data (BAP1), set to demonstrate the resolution limitations of Airyscan, however, better than confocal, in determining localization and structural details.

Summary

Panel A shows a single Airyscan optical section (z-plane 84 of 171) depicting BAP1 and H2AX distribution following etoposide-induced DNA damage. Rabbit anti-Human BAP1 Polyclonal, IgG is detected with the Identifyn® Goat anti-Rabbit 594 secondary antibody (red), while Mouse anti-Human H2AX Monoclonal, IgG1 – (1H1D9/B7) is detected with the Identifyn® donkey anti-mouse 488 secondary antibody (green). Following damage induction, BAP1 demonstrates focal nuclear recruitment to H2AX-associated damage-responsive regions, consistent with chromatin-associated DNA repair signaling, and localized enrichment within damaged nuclear microenvironments.

Panel B shows a three-dimensional Airyscan volume projection with an X–Y cut plane (white) and X–Z cut plane (red). A region of interest (ROI) identified by the yellow circle highlights a highly conflated signal region selected for comparison with the corresponding STORM reconstruction shown in Panel C. While the Airyscan reconstruction demonstrates broad structural similarity and confirms volumetric nuclear recruitment of BAP1 into H2AX-positive regions, the signal remains diffraction-influenced. It lacks the spatial resolving power necessary to distinguish discrete nanoscale substructure. The apparent puncta and aggregate domains, therefore, likely represent clustered or overlapping repair-associated assemblies rather than resolved molecular architecture. Although Airyscan provides substantial improvement over conventional confocal microscopy through enhanced optical sectioning and improved lateral resolution, these data demonstrate that the modality remains insufficient for definitive interpretation of nanoscale chromatin organization or repair-complex topology.

Panel C shows STORM imaging of the corresponding region of interest, revealing sub-diffraction structural organization not resolved in the Airyscan reconstruction. While broad signal distribution patterns between modalities show vague structural similarity, the STORM dataset demonstrates substantially greater spatial separation and architectural refinement, highlighting the conflated nature of the Airyscan signal at this scale. Together, these observations demonstrate that lower-resolution modalities can

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reliably identify biologically relevant recruitment and nuclear localization patterns; however, accurate interpretation of nanoscale repair architecture requires true super-resolution imaging approaches.

Biologically, BAP1 is a chromatin-associated tumor suppressor deubiquitinase involved in DNA double-strand break repair, chromatin remodeling, and homologous recombination-associated signaling. Following genotoxic stress, BAP1 is recruited into damage-associated nuclear regions, where it participates in the regulation of H2A ubiquitination and in the stabilization of repair complexes. H2AX serves as a broad chromatin-associated marker of DNA damage signaling following double-strand break formation. The observed spatial overlap between BAP1 and H2AX is therefore consistent with coordinated recruitment into DNA damage response microenvironments, though spatial proximity at diffraction-limited resolution does not imply direct molecular interaction.

Image Correction

NONE

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

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