

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 \(cat#P36962\)](#).

H2Ax Acquired, however, not shown in Data Analysis - Focus on BAP1 Polyclonal Structural Details.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) -

Z-Stack = 117 Slices (3.48 μ m)- 2D at Z-Plane 41 of 117, Panel A

Channels = 2

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 1593 X 1891

Image Size (Scaled) = 33.63 μ m X 38.92 μ m
Bit Depth = 16 Bit
Image Center Position = X: 61.24 mm, Y: 40.07 mm
Stage Position = X: 61.24 mm, Y: 40.07 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Region of Interest Panels A, Tomo3D Presentation**Z-Stack = 117 Slices (3.48 μ m)**

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 449 X 447
Image Size (Scaled) = 9.48 μ m X 9.44 μ m
Bit Depth = 16 Bit
Image Center Position = X: 61.24 mm, Y: 40.07 mm
Stage Position = X: 61.24 mm, Y: 40.07 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

594 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.92 μ m
Binning Mode = 2,2
Laser Wavelength = 561 nm @ 1.50%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 32.5 μ m grating

488 - Acquired but not shown in Data Analysis.

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525

Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = AxioCam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 μ m
Binning Mode = 2,2
Laser Wavelength = 488 nm @ 1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 μ m grating

Post Processing - SIM2 Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = 4
Channels = 2
Algorithm = SIM2
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = 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), with a region of interest denoted by a white box. Image is at z-plane 41 of 117

Tomo3D - Panel B

Ortho Display

Cut Lines - X = 225, Line Width 22, Y = 224, Line Width 22, Z = 24, Line Width 24
Line Opacity = 100

3D Display

3D Transparency Projection = Precise
Appearance = Threshold 13%, Ramp 20%, Maximum 100%
Background = Black
Light = Brightness 100%

STORM - Panel C

STORM image shown from the same product data (BAP1), set to demonstrate the resolution improvements of Lattice SIM Squared vs. STORM.

Summary

Rabbit anti-Human BAP1 Polyclonal, IgG was visualized using Identifyn® SRM Alexa Fluor™ 594 affinity-purified Goat anti-Rabbit IgG (H + L), with all Alexa Fluor™ 594 signal pseudocolored white from the default red to improve visualization and structural interpretation throughout the dataset.

Panel A shows a Lattice SIM² optical section at Z-plane 41 of 117 following etoposide-induced DNA damage, demonstrating a strong aggregate nuclear response consistent with redistribution of BAP1 into damage-associated repair domains. A white boxed region identifies the area of interest further explored in Panel B.

Panel B presents a Tomo3D orthogonal reconstruction generated using Zeiss ZEN software. The upper left panel shows the 3D maximum projection of the region of interest derived from Panel A. The blue mask represents the Z-position of the orthogonal section and is additionally indicated by the blue line shown in both the upper right and lower left panels. The lower left panel corresponds to the original Z-plane from Panel A, where orthogonal crosshairs define the region selected for volumetric analysis. The upper right panel represents the two-dimensional XZ orthogonal view corresponding to the red crosshair shown in the lower left image, with Z-position indicated by the blue line. The lower right panel shows the ZY orthogonal reconstruction corresponding to the green crosshair, likewise referenced to the same Z-position.

Within the ZY reconstruction, an unresolved triplet-like organization is visible. Although affected by expected Z-axis aberration, spatial separation between structures remains evident. Similarly, the XZ orthogonal view demonstrates a partially resolved doublet configuration, again limited by axial distortion but still suggestive of underlying structural separation. While Lattice SIM² substantially improves structural resolution approaching ~40 nm in the XY plane, interpretation remains biased toward the higher-confidence structural ground truth provided by STORM imaging. Nevertheless, the concordance observed between Airyscan, Lattice SIM, and Lattice SIM² suggests that these super-resolution modalities are capturing related subnuclear organizational features despite incomplete axial resolution. As such, these approaches maintain an important role within the Stepdown Methodology™, particularly in colocalization workflows and in the progressive unwinding of conflated fluorescence signals.

Image Correction

NONE

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.