

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P)***Cat ID# PA-RR 000014***Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)***Cat ID# - PA-MM 000002*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000015*

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

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 20 hours, followed by -20°C methanol fixation and blocking with [Thermo Fisher Fish Serum Blocker cat#37527X3](#).

The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour 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 5X in 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 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](#).

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 81 of 181

Z-Stack = 181 Slices (5.40 μ m)

Channels = 3

Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm

Image Size (Pixels) = 870 X 773

Image Size (Scaled) = 30.70 μ m X 27.78 μ m

Bit Depth = 16 Bit

Image Center Position = X: 68.96 mm, Y: 49.41 mm

Stage Position = X: 68.96 mm, Y: 49.41 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Panel B

Z-Stack = 181 Slices (5.40 μ m)

Channels = 3

Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm

Image Size (Pixels) = 93 X 92

Image Size (Scaled) = 3.28 μ m X 3.25 μ m

Bit Depth = 16 Bit

Image Center Position = X: 68.96 mm, Y: 49.41 mm

Stage Position = X: 68.96 mm, Y: 49.41 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

647 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 5.00 AU / 328 μ m

LMS MBS = MBS 488/639 & 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 = Super Resolution: 10.0 (3D, Auto)

488 - *Acquired but not Shown in Data Analysis.*

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = **488 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 = 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 = Super Resolution: 10.0 (3D, Auto)

DAPI - *Acquired but not Shown in Data Analysis.*

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 214 μm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **405 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 = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 8.2 (3D, Auto)

2D View - Panel A

Airyscan optical section acquired at Z-plane 81 of 181 showing Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG – (BLR038P), detected using Identifyn® SRM Alexa Fluor™ 647 affinity-purified Goat Anti-Rabbit, IgG (H + L) (red). A white box denotes the region of interest (ROI) selected for further structural comparison in Panels B and C.

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
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)

STORM - Panel C

The corresponding STORM dataset was generated from the same BAP1.p (Serine 592) and is shown to demonstrate the resolution limitations of Airyscan while also highlighting its improved structural detail and localization capability relative to conventional confocal microscopy. Although Airyscan provides enhanced visualization of phospho-BAP1-associated aggregate organization, the observed structures remain partially diffraction-limited compared with the nanoscale resolution achieved by single-molecule localization microscopy using STORM.

Summary

Panel A depicts Airyscan super-resolution fluorescence microscopy acquired at Z-plane 81 of 181, demonstrating Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit secondary antibody following etoposide-induced DNA damage. Although H2Ax-associated signal collected using the 488 channel and DAPI nuclear staining were simultaneously acquired during image collection, these channels were intentionally omitted from the present analysis to focus specifically on phospho-BAP1 structural organization and to facilitate direct comparative assessment against the corresponding STORM dataset. A white boxed region of interest is highlighted within Panel A to identify a phospho-BAP1-associated aggregate region selected for further structural examination in Panel B. Consistent with the preceding widefield, widefield SIM, and confocal datasets, phosphorylated BAP1 demonstrated pronounced nuclear redistribution with the formation of discrete punctate aggregates and chromatin-associated signaling domains throughout the nucleus following DNA damage induction.

Panel B shows a processed 3D volume rendering in which the Z-stack was computationally collapsed to approximately 50% of its original volume to reduce axial aberration and improve visualization of recurring phospho-BAP1-associated morphologic features within the Airyscan dataset. A circled region of interest is highlighted and connected by a directional arrow to the corresponding STORM image shown in Panel C to facilitate qualitative structural comparison between the two imaging modalities. While the Airyscan reconstruction begins to demonstrate improved subcellular detail and aggregate organization beyond that achievable with conventional confocal microscopy, the observed structures remain partially diffraction-limited and lack the nanoscale structural resolving power demonstrated in the corresponding STORM acquisition.

Importantly, although Airyscan approximately doubles lateral resolution relative to traditional confocal imaging through detector-array reassignment and computational deconvolution approaches, the modality remains fundamentally constrained in its ability to resolve the nanoscale substructures and aggregate-associated architectural organization visualized using STORM. As such, while broad morphologic similarities between the Airyscan and STORM datasets may be appreciated within selected regions, direct one-to-one structural interpretation should be approached cautiously, as the apparent Airyscan-associated aggregate morphology likely represents unresolved composite signal originating from multiple underlying nanoscale phospho-BAP1-associated repair domains. These findings remain highly consistent with the progressive resolution hierarchy observed throughout the preceding stepdown datasets, collectively demonstrating how increasing optical resolving power incrementally reveals additional levels of phospho-BAP1 spatial organization within DNA damage-associated chromatin microenvironments.

Image Correction

NONE

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

