

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

Z Stack - (3D) - Panel A, Z-Plane 85 of 177

Z-Stack = 177 Slices (5.28 μ m)

Channels = 2

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

Image Size (Pixels) = 1927 X 2049

Image Size (Scaled) = 40.68 μ m X 43.26 μ m

Bit Depth = 16 Bit

Image Center Position = X: 66.86 mm, Y: 36.62 mm

Stage Position = X: 66.86 mm, Y: 36.62 mm

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

Z Stack - (3D) - Panel B, Volume Projection

Z-Stack = 80 Slices (2.37 μ m) - Displayed gallery content was reduced to Phospho-BAP1-specific signal.

Channels = 2

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

Image Size (Pixels) = 295 X 311

Image Size (Scaled) = 6.23 μ m X 6.57 μ m

Bit Depth = 16 Bit

Image Center Position = X: 66.86 mm, Y: 36.62 mm

Stage Position = X: 66.86 mm, Y: 36.62 mm

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

647 -

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 = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1x
Exposure Time = 100 ms
Depth of Focus = 1.13 μm
Binning Mode = 2,2
Laser Wavelength = 640 nm @ 1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 μm grating

488 -

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 = 503 - 546, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1x
Exposure Time = 120 ms
Depth of Focus = 0.81 μm
Binning Mode = 2,2
Laser Wavelength = 488 nm @ 1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 μm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard

Auto Sharpness = On
 Sharpness = 10.3458 (647), 11.5304 (488)
 Fitting = Fast Fit
 Normalization = Auto

2 Dimensional View - Panel A

Shows an Elyra 5 Lattice SIM optical section acquired at Z-plane 85 of 177, demonstrating Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG – (BLR038P), visualized using Identifyn® SRM Alexa Fluor™ 647 Goat anti-Rabbit secondary antibody, and Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 Donkey anti-Mouse secondary antibody. A white box denotes the region of interest (ROI) selected for further examination in Panel B.

3D Image Processing - Panel B, Showing Phospho-BAP1/647 only

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 75%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Plane Process - Panel B

Clipping planes are introduced in Panel B to highlight the Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P) within the nuclear compartment by adjusting or cutting away portions of the image using wedge cut processing, removing parts of the image in the X-Z and Y-Z planes.

X-Z = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -30%

Clip Y = -15°

Clip Z = 0°

Y-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 = -36%

Clip Y = 15°

Clip Z = 0°

Panel B shows a wedge-cut volume-rendered view from the top with a slight backward rotation, highlighting the wedge-removal region and revealing BAP1.p (Serine 592)-associated signal distribution throughout the z-stack. This orientation provides XZ and YZ visualization planes for structural comparison against the corresponding STORM dataset.

STORM - Panel C

The corresponding STORM dataset was generated from the same BAP1.p (Serine 592) and is shown to demonstrate the enhanced nanoscale structural resolution achieved relative to the corresponding Lattice SIM dataset, while also highlighting the overall

consistency in BAP1.p-associated aggregate morphology and spatial organization observed between the two imaging modalities.

Summary

Panel A depicts Lattice SIM super-resolution fluorescence microscopy acquired at Z-plane 85 of 177, demonstrating 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), together with H2Ax detected using Identifyn® SRM Alexa Fluor™ 488 affinity-purified Donkey Anti-Mouse, IgG (H + L). A white box denotes the region of interest (ROI) selected for further structural examination in Panel B. Consistent with the preceding widefield, widefield SIM, confocal, and Airyscan datasets, phosphorylated BAP1 demonstrated pronounced nuclear redistribution with the formation of discrete punctate aggregates and DNA damage-associated chromatin signaling domains following etoposide-induced DNA damage.

Panel B shows a processed three-dimensional volume-rendered reconstruction of the ROI identified in Panel A. For visualization purposes, the phospho-BAP1-associated 647 signal was pseudocolored white rather than the default red to enhance visual contrast and improve assessment of aggregate-associated structural organization. The H2Ax-associated channel was omitted from the processed rendering to focus specifically on phospho-BAP1 morphology and facilitate direct comparison with the corresponding STORM dataset shown in Panel C. Wedge-view processing was employed using both X-Z and Y-Z clipping planes, with the X-Z plane pseudocolored green and the Y-Z plane pseudocolored red. The cut planes were positioned at approximately 15-degree angles to expose the internal Z-axis structural organization and to generate a volumetric perspective that closely matches the Y-Z subset visualization shown in the lower-right region of the corresponding STORM image.

Importantly, Lattice SIM demonstrated substantial improvements in evaluating phospho-BAP1 localization, aggregate organization, and volumetric structural distribution compared with prior imaging modalities. While the modality does not fully achieve the nanoscale resolution of STORM, both the XY and YZ subsets demonstrated recurring morphological similarities and consistent aggregate-associated organizational patterns across the two imaging platforms. These findings suggest that Lattice SIM provides a biologically consistent intermediate-resolution representation of phospho-BAP1-associated repair domains, enabling substantially improved structural interpretation while preserving broader contextual nuclear architecture that may become fragmented or abstracted in single-molecule localization microscopy datasets such as STORM.

Image Correction

NONE

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

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