

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) = 1988 X 2024

Image Size (Scaled) = 41.97 μ m X 42.73 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, 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) = 426 X 403

Image Size (Scaled) = 8.99 μ m X 8.51 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 C, Three Dimensional Volume View

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) = 132 X 125

Image Size (Scaled) = 2.79 μm X 2.64 μm
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 μm , Y: 0.00 μm

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 - 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 an Elyra 5 Lattice SIM2 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.

2 Dimensional View - Panel B

Region of interest from Panel A showing phospho-BAP1/647 pseudocolored white to enhance structural contrast. A white box denotes an additional ROI for further examination in Panel C.

3D Image Processing - Panel C

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)

Summary

Panel A depicts Zeiss Elyra 5 Lattice SIM² 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, Airyscan, and Lattice SIM datasets, phosphorylated BAP1 demonstrated pronounced nuclear redistribution with the formation of discrete punctate aggregates and chromatin-associated DNA damage response domains following etoposide-induced DNA damage.

Panel B shows a high-resolution 2D view 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 improve visual contrast and facilitate assessment of fine structural organization. Within this region, a secondary ROI highlighting a representative phospho-BAP1-associated structural assembly was selected for further volumetric reconstruction and examination in Panel C.

Panel C shows a processed three-dimensional volume-rendered reconstruction of the ROI identified in Panel B. Unlike the preceding Airyscan and Lattice SIM datasets, direct comparison with STORM was intentionally omitted in this dataset because the observed volumetric structural organization demonstrated highly recurring and reproducible morphologic patterns consistently identified across thousands of DNA damage response-associated protein datasets analyzed using the Identifyn® stepdown framework. At the resolution achieved using Lattice SIM², chromatin-associated structural assemblies become readily visible throughout the nuclear volume, demonstrating reproducible heterogeneous chromatin-associated patterning across phospho-BAP1-associated repair domains.

Importantly, while STORM continues to provide the highest structural resolution and approximate ground-truth localization performance at ~20 nm resolution, Lattice SIM² offers substantial advantages in volumetric structural interpretation, achieving ~40 nm lateral resolution in the X and Y axes while preserving highly coherent three-dimensional contextual architecture across extended Z-stack acquisitions. As such, Lattice SIM² provides a complementary super-resolution perspective distinct from STORM, enabling improved visualization of chromatin-associated volumetric repair structures and heterogeneous nuclear organizational patterning that may be less readily appreciated within highly localized single-molecule reconstruction datasets alone.

Image Correction

NONE

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

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