

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 Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3

Scaling (Per Pixel) = 0.110 μ m X 0.110 μ m

Image Size (Pixels) = 1126 X 954

Image Size (Scaled) = 123.32 μ m X 104.49 μ m

Bit Depth = 14 Bit

Image Center Position = X: 0.00 μ m, Y: 0.00 μ m

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

647 -

Reflector = 50 Cy 5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715

Contrast Method = Fluorescence

Light Source = X-Cite Xylis Lamp Intensity @ 15.00%

Exposure Time = 150 ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.4

Imaging Device = Axiocam 705

Camera Adapter = 1X

Depth of Focus = 1.03 μ m

Binning Mode = 2,2

488 -

Reflector = 38 eGFP

Beam Splitter = 495

Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light Source = X-Cite Xylis Lamp Intensity @ 20.00%
 Exposure Time = 200 ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Imaging Device = AxioCam 705
 Camera Adapter = 1X
 Depth of Focus = 0.80 μm
 Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335 - 383
 Filter Emission Wavelength = 420 - 470
 Contrast Method = Fluorescence
 Light Source = X-Cite Xylis Lamp Intensity @ 5.00%
 Exposure Time = 20 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = AxioCam 705
 Camera Adapter = 1X
 Depth of Focus = 0.72 μm
 Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG – (BLR038P), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit IgG (H + L). The top-right panel shows Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse IgG (H + L). The lower-left panel shows DAPI nuclear staining, and the bottom-right panel shows the three-channel merged image.

Colocalization View - Panel B

Horizontal Axis - AF647, Threshold 95, Range Auto - Phospho-BAP1 (Serine 592)
 Vertical Axis - AF488, Threshold 256, Range Auto - H2Ax

Image Measurement = Entire Field

Colocalization analysis of Phospho-BAP1 (X-axis) and H2Ax (Y-axis) was performed across the entire image acquisition.

Summary

Panel A shows a split-view widefield acquisition following etoposide-induced DNA damage. The upper left panel shows 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). The upper right panel shows Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7), detected using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse IgG (H + L). The lower-left panel shows DAPI nuclear staining, while the lower-right panel shows the three-channel merged image. Following etoposide exposure, phospho-BAP1 exhibits prominent intranuclear redistribution, with punctate aggregate formation and enrichment in damage-associated nuclear regions. The H2Ax signal remains broadly distributed throughout the nucleus, with an overlapping organization within DNA damage-responsive chromatin territories.

Panel B presents colocalization analysis of the same acquisition, with phospho-BAP1 plotted along the horizontal (X) axis and H2Ax plotted along the vertical (Y) axis. Broad overlapping localization patterns are observed between the two signals, consistent with coordinated engagement of DNA damage-associated nuclear compartments following genotoxic stress.

Biologically, BAP1 (BRCA1-associated protein 1) functions as a nuclear deubiquitinase involved in chromatin remodeling, DNA damage signaling, homologous recombination repair, and transcriptional regulation. Following DNA damage, phosphorylated BAP1 is recruited to chromatin-associated repair environments where it contributes to the regulation of repair complex assembly and chromatin accessibility. Etoposide-induced DNA double-strand breaks stimulate the recruitment of the DNA damage response machinery, including ATM/ATR-associated signaling pathways, which influence BAP1 phosphorylation state and localization dynamics.

H2Ax serves as a core chromatin-associated histone variant distributed broadly throughout the nucleus and functions as the substrate pool for phosphorylation into γH2AX following DNA damage induction. As such, overlapping spatial distributions between phospho-BAP1 and total H2Ax are expected within damage-responsive chromatin regions, particularly at widefield resolution, where diffraction-limited imaging conflates densely organized nuclear structures.

Importantly, while widefield microscopy effectively demonstrates global nuclear recruitment, aggregate formation, and apparent colocalization patterns, it remains insufficient to resolve nanoscale structural organization or to directly resolve molecular interactions. The strong overlap observed between phospho-BAP1 and H2Ax should therefore be interpreted as coordinated localization within shared DNA damage response territories rather than definitive molecular colocalization. Higher-resolution modalities remain necessary to resolve the underlying sub-diffraction architecture of these repair-associated signaling domains.

Image Correction

NONE

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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