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.

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Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, DNA damage was induced by treatment with 2 µM 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, Showing Z-Plane 63 of 181*
Z-Stack = 181 Slices (5.40 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1503 X 1503
Image Size (Scaled) = 88.38 µm X 88.38 µm
Bit Depth = 16 Bit
Image Center Position = X: 74.45 mm, Y: 46.33 mm
Stage Position = X: 74.44 mm, Y: 46.33 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 65 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = **639 nm @ 0.4%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26 µs
Frame Time = 5.63 s
LSM Scan Speed = 11
Scan Direction = Bidirectional

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Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.5 (3D, Auto)

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

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 51 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **488 nm @ 0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26 µs
Frame Time = 5.63 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.8 (3D, Auto)

DAPI -
Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = **405 nm @ 0.4%**
Laser Blanking = Enabled
Scan Mode = Frame

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Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26 µs
Frame Time = 5.63 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 435 - 490
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.8 (3D, Auto)

Two-Dimensional View - *Panel A*

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), together with DAPI nuclear staining. A line profile is drawn across the nucleus for quantitative assessment of fluorescence intensity in Panel B.

Profile View Display - *Panel B*

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 63.2), Y (Min 0.0 and Max 34297)

Measured at Z-plane 63 of 181

Zen Acquisition Info Tab - *Panel C*

Shown are the acquisition parameters for the 647 channel corresponding to BAP1.p (Serine 592) and the DAPI channel, collected using the 639 nm and 405 nm lasers at 0.4% power, respectively. Scan zoom, scan speed, and line-averaging settings are provided to ensure transparency, reproducibility, and consistency of the fluorescence line-profile measurements acquired from the BAP1.p (Serine 592) nuclear aggregates.
The 488 channel (H2Ax) is also shown at 0.2% but was not used in the data analysis.

Summary

Panel A shows an LSM 980 confocal optical section acquired at Z-plane 63 of 181, demonstrating Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit secondary antibody, together with DAPI nuclear staining. A line profile traversing the nucleus measures fluorescent intensity across both channels, demonstrating that the

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BAP1.p (Serine 592) signal remains predominantly confined to the nuclear compartment. The line profile additionally reveals multiple punctate regions of elevated BAP1.p (Serine 592) intensity associated with intranuclear aggregate formation following etoposide-induced DNA damage.

Panel B shows quantitative analysis of the fluorescence intensities measured from the line profile shown in Panel A. Three prominent BAP1.p (Serine 592)-associated aggregate regions were identified with measured fluorescent intensities of 32,664.00, 26,333.00, and 22,050.00 arbitrary units (A.U.), respectively. These findings support localized redistribution and concentration of phosphorylated BAP1 into discrete intranuclear signaling domains rather than a uniform increase in total nuclear-associated fluorescence. While BAP1 is constitutively present throughout the nucleus under basal conditions, genotoxic stress promotes phosphorylation-dependent spatial reorganization into punctate, chromatin-associated repair microenvironments that are involved in DNA damage response signaling and chromatin remodeling.

Panel C provides acquisition transparency by including the Zeiss ZEN imaging parameters used to collect the dataset. Recorded excitation inputs included 0.4% laser power for the 639 nm channel corresponding to Alexa Fluor™ 647 and 0.4% laser power for the 405 nm DAPI channel. See Full Summary Methods Section.

Image Correction

NONE

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

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