

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*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057*

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**Plus Damage -**

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 3 times in 1X 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 5 times in 1X PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI \(cat# P36962\)](#).

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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 = 1

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

Z Stack - (3D) - Panel B, Showing Z-Plane 81 of 161

Z-Stack = 161 Slices (4.80 µm)

Channels = 1

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image Size (Pixels) = 1248 X 1248

Image Size (Scaled) = 73.41 µm X 73.41 µm

Bit Depth = 16 Bit

Image Center Position = X: 70.82 mm, Y: 52.34 mm

Stage Position = X: 70.82 mm, Y: 52.34 mm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 - Plus Etoposide, Upper Panel

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
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)

647 - No Etoposide, Lower Panel - Control

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 @ 0.6%** -

A higher image input setting was required in the untreated control sample to visualize the absence of a detectable phospho-BAP1-associated signal.

Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.27 µs
Frame Time = 4.10 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
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: 8.9 (3D, Auto)

Profile View Display - *Plus DNA Damage, Top*

Profile Definition = Stroke Thickness 1.00, 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)**

This panel measures Phospho-BAP1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately 40K A.U. at its maximum.

Measured at the Z-plane, 68 of 181.

Profile View Display - *Minus DNA Damage, Bottom*

Profile Definition = Stroke Thickness 1.00, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - **X (Min 0.0 and Max 46.2), Y (Min 0.0 and Max 1117)**

This panel measures Phospho-BAP1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~3K A.U. at its maximum.

Measured at the Z-plane, 81 of 161.

Histogram Display - *Plus DNA Damage, Top*

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 2000000)

Arithmetic Mean intensity = 1,400.456

The histogram was generated from the entire image field to quantify the overall Phospho-BAP1 response following DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - *Minus DNA Damage, Bottom*

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 2000000)

Arithmetic Mean intensity = 9.616

The histogram was generated from the entire image field to quantify the overall Phospho-BAP1 protein level in the absence of DNA damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Summary

In untreated cells, the phosphorylated BAP1 signal was effectively absent, with only minimal background-associated fluorescence observed throughout the nucleus. This finding is consistent with the tightly regulated nature of BAP1 phosphorylation and supports the expectation that phospho-BAP1-associated signaling events occur predominantly following genotoxic stress and activation of the DNA damage pathway. In the untreated control population, no meaningful punctate nuclear aggregates or damage-associated signaling domains were identified, and the residual low-level signal appeared diffuse and non-structured in distribution. Due to the near-complete absence of detectable phospho-BAP1 signal in these control cells, the range indicator was activated in the lower line-profile image to demonstrate cellular positioning and confirm that the absence of signal corresponded to true biological signal loss rather than the absence of cellular material within the sampled region.

Following etoposide-induced DNA damage, phosphorylated BAP1 showed a pronounced increase in both overall nuclear-associated fluorescence intensity and focal signal enrichment, with the formation of discrete nuclear aggregates consistent with DNA damage-associated chromatin signaling domains. Quantitative analysis showed an arithmetic mean fluorescent intensity of **1,400.456 A.U.** in the damaged population, compared with **9.616 A.U.** in untreated cells, representing an approximate **145.64-fold increase** in the phosphorylated BAP1-associated signal following DNA damage induction. Line-profile and histogram analysis further supported this marked redistribution and enrichment response, with maximal aggregate intensity increasing from **2,845.000 A.U.** in untreated cells to **42,144.000 A.U.** following etoposide exposure, corresponding to an approximate **14.8-fold increase** in peak focal signal intensity.

Biologically, these findings are consistent with phosphorylation-dependent recruitment and stabilization of BAP1 within DNA damage response-associated chromatin microenvironments following double-strand break induction and replication-associated stress signaling. The observed punctate enrichment pattern likely reflects clustered repair-associated signaling domains and localized chromatin-remodeling events rather than isolated molecular assemblies. At diffraction-limited resolution, focal overlap with additional DNA damage response-associated proteins should be interpreted as coordinated pathway engagement within shared nuclear repair microenvironments rather than as direct evidence of molecular interaction.

Image Correction

The phospho-BAP1 channel (647) was displayed in grayscale to enhance visual contrast.

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