

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human BAP1.p Polyclonal, IgG***Cat ID# - PA-RP 000005*Identifyn® Secondary Antibody**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)***Cat ID# - SA 000063*

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR. However, its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. Phosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

Plus Damage – Panel A

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide–spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with [Thermo Fisher Fish Serum Blocker cat#37527X3](#). The primary antibody, Rabbit anti-Human BAP1.p antibody, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI cat#P36962](#).

No Damage – Panel B

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with [Thermo Fisher Fish Serum Blocker cat#37527X3](#). The primary antibody, Rabbit anti-Human BAP1.p antibody, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour 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 Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D) – Panel A (Z-plane 21 of 44)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm
Image size (Pixels) = 761 X 707
Image Size (Scaled) = 108.71µm X 101.00µm
Bit Depth = 14 Bit
Image Center Position = X: 48.10µm, Y: 49.96µm
Stage Position = X: 48.10µm, Y: 49.96µm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

Single Plane (2D) – Panel B (Z-plane 21 of 44)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm
Image size (Pixels) = 1080 X 741
Image Size (Scaled) = 152.29µm X 105.86µm
Bit Depth = 14 Bit
Image Center Position = X: 70.95µm, Y: 44.96µm
Stage Position = X: 70.95µm, Y: 44.96µm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

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 @6.00%
 Exposure Time = 40 ms
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.25
 Imaging Device = 807 mono
 Camera Adapter = 1X
 Depth of Focus = 1.30 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 6.00%
 Exposure Time = 18 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.25
 Imaging Device = 807 mono
 Camera Adapter = 1X
 Depth of Focus = 0.90 μm
 Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mod" "Optical Sectioning" - enabled correction selected.

Normalization, Phase Corrector, Fourier Filter, and Deconvolution **were NOT used.**

Summary

Panel A - DNA damage was induced by treating cells with 2 μM etoposide-spiked complete growth media for 24 hours. This panel presents a split-channel view of z-plane 21 out of 44, which was used for both Panel A and Panel B. The left-hand panel shows the Rabbit anti-Human BAP1.p antibody, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary (red), while the middle panel displays the DAPI nuclear staining (blue). The right panel merges both channels. The introduction of DNA damage demonstrates a discernible response, evidenced by the formation of pan-nuclear staining for BAP1.p. Notably, the protein's absence in the nucleolar space is expected. Furthermore, while widefield SIM is diffraction-limited, we observe the formation of aggregates or foci, which we intend to investigate further using our step-down methodology, particularly through super-resolution Airyscan, SIM, SIM2, and STORM techniques.

Panel B – DNA damage was not induced in this panel. It presents a split-channel view of the two-dimensional image collected. The left-hand panel again shows the Rabbit anti-Human BAP1.p antibody, IgG, counterstained with Identifyn[®] SRM Alexa Fluor[™] 647 secondary (red). In contrast, the middle panel displays the DAPI nuclear staining (blue). The right panel merges both channels. The lack of DNA damage results in little to no BAP1.p signal being detected. Although there is some negligible signal under the same imaging conditions, it does not match the robust protein aggregation observed following DNA damage. This leads us to conclude that BAP1.p was activated and recruited following DNA damage and is strongly localized within the nuclear compartment.

Panel C – This panel displays the acquisition settings used for both images, confirming that they are identical, with the same excitation input and exposure times. Additionally, we note that the image acquisition was performed in a z-stack, using the same z-plane for both cases.

Image Corrections

None

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