

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human BAP1.p Polyclonal, IgG***Cat ID# - PA-RP 000005*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)***Cat ID# - SA 000063***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific***Cat ID# - SA 000012*

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, although 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

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M 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 is Rabbit anti-Human BAP1.p Polyclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), 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 Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:100. 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) = 1216 X 1028
 Image Size (Scaled) = 133.18 μ m X 112.59 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 @10.00%
 Exposure Time = 100 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 @10.00%
 Exposure Time = 100 ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Imaging Device = AxioCam 705
 Camera Adapter = 1X
 Depth of Focus = 0.80 μ m
 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 @15.00%
 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 μ m
 Binning Mode = 2,2

Split View - Panel A

The top-left panel features Rabbit anti-Human BAP1.p Polyclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L), the top-right panel shows Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fc γ Fragment Specific, the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels.

Colocalization View - Panel B

Horizontal Axis - AF647, Threshold 8, Range Auto
 Vertical Axis - DAPI, Threshold 191, Range Auto

Summary

Panel A - Split View illustrates the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG. The upper left section features the Rabbit anti-Human BAP1.p Polyclonal IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right displays an Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). The lower left section shows DAPI nuclear staining (blue). In contrast, the lower right presents a merged image of the three channels.

Panel B – Displays a colocalization view of the DAPI (Y-axis) and Rabbit anti-Human BAP1.p Polyclonal IgG with an Identifyn® SRM Alexa Fluor™ 647 channel (X-axis), showing that BAP1.p is strongly colocalized with DAPI and is thereby, via this 2-dimensional image, within the nuclear compartment as expected for this protein following Etoposide-induced DNA damage.

Image Correction

None

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Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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