

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™ 555 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000042*

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

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, [Nuclear Pore Complex Monoclonal Antibody \(39C7\)](#), 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™ 555 Affinity Purified Donkey Anti-Mouse, F(a'')₂ (H + L) 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 Imager.M1 Widefield, 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) - Z-plane 24 of 91 total - Panel A

Z-Stack = 91 Slices (9 μ m)

Channels = 3
Scaling (Per Pixel) = 0.143 μ m X 0.143 μ m X 0.100 μ m
Image Size (Pixels) = 572 X 573
Image Size (Scaled) = 81.71 μ m X 81.86 μ m
Bit Depth = 14 Bit
Image Center Position = X: 49.39 mm, Y: 51.35 mm
Stage Position = X: 49.39 mm, Y: 51.35 mm
ROI Center Offset = X: 1.14 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 @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

555 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 120 ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.10 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 = 20 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.**

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(a'')₂ (H + L), the top-right panel shows Nuclear Pore Complexes visualized with Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)

The bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels. This image was taken at Z-Position 24 of 91.

Profile View Display - Panel B & C

Z-Position = 24 of 91

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 62.4), Y (Min 0 and Max 554)

Summary

Panel A - This panel showcases a split view illustrating the signal and localization of Rabbit anti-HumanBAP1.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. The upper right displays a Nuclear Pore Complex (NPC) primary antibody tagged with Identifyn® SRM Alexa Fluor™ 555. The lower left section shows DAPI nuclear staining (in blue), while the lower right presents a merged image of the three channels.

Panel B - This panel features a line profile display that demonstrates localization and fluorescent intensity. The X-axis represents distance, and the Y-axis represents intensity. The data indicate that the signals for Rabbit anti-Human BAP1.p Polyclonal IgG and DAPI nuclear staining occupy the exact location along the X-axis. This finding demonstrates that Rabbit anti-Human BAP1.p Polyclonal IgG and DAPI on Z-plane 24 of 91 are colocalized, occupying the same space in the image. The Y-axis shows the Rabbit anti-Human BAP1.p Polyclonal IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647, displays comparable fluorescent brightness to DAPI.

Panel C - Displays the z-plane 24 for both the 647 (BAP1.p) and DAPI channels. A line is drawn across two nuclei to illustrate the source of our measurements in panel B.

In conclusion, the Rabbit anti-Human BAP1.p Polyclonal IgG is both nuclear in response to etoposide-induced DNA damage and exhibits excellent localization and signal intensity.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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