

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. 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, [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 1:1000. Cells were washed 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI cat#P36962](#).

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 62 of 162 total - Panel A

Z Stack - (3D)

Z-Stack = 162 Slices (4.83 μ m)

Channels = 3
 Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
 Image Size (Pixels) = 4950 X 4321
 Image Size (Scaled) = 104.51 μ m X 91.23 μ m
 Bit Depth = 16 Bit
 Image Center Position = X: 56.44 mm, Y: 35.21 mm
 Stage Position = X: 56.44 mm, Y: 35.21 mm
 ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Panel B & C

Z-Stack = 162 Slices (4.83 μ m)

Channels = 3
 Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
 Image Size (Pixels) = 1246 X 1553
 Image Size (Scaled) = 26.31 μ m X 32.79 μ m
 Bit Depth = 16 Bit
 Image Center Position = X: 56.44 mm, Y: 35.21 mm
 Stage Position = X: 56.44 mm, Y: 35.21 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Panel D

Z-Stack = 162 Slices (4.83 μ m)

Channels = 3

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 555 X 548

Image Size (Scaled) = 11.72 μ m X 9.67 μ m

Bit Depth = 16 Bit

Image Center Position = X: 56.44 mm, Y: 35.21 mm

Stage Position = X: 56.44 mm, Y: 35.21 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

647 -

Reflectors = SR HE LBF 405/488/561/642

Beam Splitter = 405, 488, 561, 642

Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642

Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Contrast Method = Fluorescence

Illumination Wavelength = 640

Channel Name = T1-Axiocam 820-SR

Excitation Wavelength = 640

Emission Wavelength = 729

Effective NA = 1.4

Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Imaging Device = Axiocam 820

Camera Adapter = 1X

Exposure Time = 120 ms

Depth of Focus = 1.13 μ m

Binning Mode = 2,2

Laser Wavelength = 640 nm @1.00%

Frame Time = 1.33 s

Scan Direction = Unidirectional

Grating = 36.5 μ m grating

488 -

Reflectors = SR HE LBF 405/488/561/642

Beam Splitter = 405, 488, 561, 642

Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642

Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Contrast Method = Fluorescence

Illumination Wavelength = 488

Channel Name = T2-Axiocam 820-SR

Excitation Wavelength = 488

Emission Wavelength = 525

Effective NA = 1.4

Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Imaging Device = Axiocam 820

Camera Adapter = 1X
 Exposure Time = 120 ms
 Depth of Focus = 0.81 μ m
 Binning Mode = 2,2
 Laser Wavelength = 488 nm @1.50%
 Frame Time = 1.59 s
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T3-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50 ms
 Depth of Focus = 0.69 μ m
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @3.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM Processing

Processing Dimension = 3D
 Result Sampling = 4
 Process Sampling = N/A
 Channels = 3
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 10.5081 (647), 11.1781 (488), 9.7530 (DAPI)
 Fitting = Fast Fit
 Normalization = Auto

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 Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, 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 62 of 162.

3D Image Processing - Panel B

3D Maximum Projection = Precise

Appearance = Threshold 4% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Tone Mapping Enabled

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Surface Projection = Precise

Appearance = Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30%

Background = Black

Light = Azimuth 80°, Elongation -130°, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel D

3D Volume Projection = Precise

Appearance = Threshold 4% all channels, Ramp 96% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel displays a split view at z-plane 62 of 162, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG. The upper left section highlights the primary antibody, Rabbit anti-Human BAP1.p, labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right section shows the Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). The lower left section presents DAPI nuclear staining (blue). In comparison, the lower right section features a merged image of the three channels. A white box indicates a region of interest and will be further investigated in Panels B and C.

Panel B - This panel presents a three-dimensional maximum projection of Rabbit anti-Human BAP1.p Polyclonal IgG. It is counterstained with the Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red) and the Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green), along with DAPI (blue), utilizing super-resolution SIM imaging.

Panel C - This panel displays the same z-stack as shown in Panel B, but excludes both the green and blue channels. It presents a three-dimensional surface projection of Rabbit

anti-Human BAP1.p Polyclonal IgG using a surface projection, with the 647 channel pseudo-colored from its default red to white for improved visual contrast. A region of interest is marked with a teal box and will be further investigated in subsequent panels.

Panel D - This panel showcases the region of interest indicated in Panel C, displaying a three-dimensional volume projection of Rabbit anti-Human BAP1.p Polyclonal IgG. The 647 channel is pseudo-colored from its default red to teal for enhanced visual contrast.

Note - It is evident that super-resolution SIM provides a better visualization of BAP1.p spatial localization than super-resolution Airyscan and diffraction-limited techniques. Continued observation of nuclear proteins reveals similar structures, including branches, forks, and loops with clear voids at the center. While this may represent some aspects of chromatin structure, it is not definitive. However, it is certainly intriguing and deserves more investigation. However, we make no biological claims other than that the images are unmanipulated and reproducible.

Image Corrections

Panel C - Identifyn[®] SRM Alexa Fluor[™] 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast.

Panel D - Identifyn[®] SRM Alexa Fluor[™] 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L) was pseudo-colored in Zen Software from red, the default color, to teal to provide visual contrast.

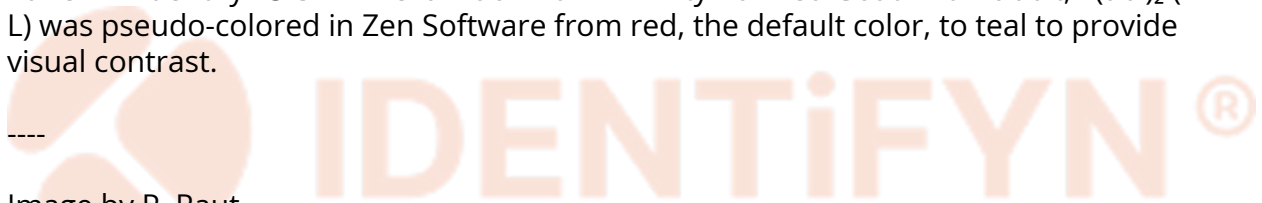


Image by P. Raut

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Data Presentation by P.D. Amistoso

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