

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 82 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) = 5056 X 5056
Image Size (Scaled) = 106.75 μ m X 106.75 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

Single Plane (2D) - Z-plane 82 of 162 total - Panel B

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) = 1406 X 1521
Image Size (Scaled) = 29.68 μ m X 32.11 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 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) = 255 X 212
Image Size (Scaled) = 5.38 μ m X 4.48 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 = 100 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 - SIM² Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM²
SIM² Settings = Standard
Input SNR = Low
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel C

3D Volume Projection = Precise

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

Background = Black

Light = Brightness 90%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 45°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel displays a 2-dimensional view at z-plane 82 of 162, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (red), Alpha Tubulin primary antibody labeled with Identifyn™SRM Alexa Fluor™ 488 (green), and DAPI nuclear staining (blue). A white box indicates a region of interest and will be further reduced and investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 82 of 162, illustrating 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). DAPI is omitted to allow for better visualization of the BAP1.p. We also note the resolution, as alpha tubulin segmentation is well-documented and reproducible, and serves as an internal control for resolution. A white box indicates a region of interest and will be further reduced and investigated in Panel C.

Panel C - This panel showcases the region of interest indicated in Panel B, 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 light grey for enhanced visual contrast.

Note - It is evident that super-resolution SIM, in this case, SIM Squared, provides a better visualization of BAP1.p spatial localization than super-resolution Airyscan and diffraction-limited techniques. Using the same sample as was imaged for SIM, we demonstrate a gain in resolving the three-dimensional structural localization details of BAP1.p recruited following etoposide-induced DNA damage to the breaks or areas of DNA breaks. Again, we make no claims as to what this image represents, as we are at ~40-60 nm in X, Y, but it is not hard to imagine that this is coating chromatin.

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 light grey to provide visual contrast.

Image by P. Raut

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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