

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 LSM 980 Confocal **with NIR Detection**, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 161 Slices (4.8 μ m)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 1566 X 1566
Image Size (Scaled) = 55.27 μ m X 55.27 μ m
Bit Depth = 16 Bit
Image Center Position = X: 84.20 mm, Y: 47.85 mm
Stage Position = X: 84.20 mm, Y: 47.85 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Single Plane (2D) - Z-plane 81 of 161 total - Panel B

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

Z-Stack = 161 Slices (4.8 μ m)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 744 X 589
Image Size (Scaled) = 26.26 μ m X 20.79 μ m
Bit Depth = 16 Bit
Image Center Position = X: 84.20 mm, Y: 47.85 mm
Stage Position = X: 84.20 mm, Y: 47.85 mm

ROI Center Offset = X: 0.00 μm , Y: 0.00 μm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 μm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS LP 640
Laser Wavelength = 639 nm @0.08%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 μs
Frame Time = 7.82 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643-735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.8 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 268 μm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS Plate 10°
Laser Wavelength = 488 nm @0.08%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 μs
Frame Time = 7.82 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493

Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499-549, 573-627
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.6 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 214 μ m
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 μ s
Frame Time = 7.82 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422-477
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.5 (3D, Auto)

3D Image Processing - Panel A

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% 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)

Clipping Image Process = Clipping planes are introduced to define regions of special interest.

X-Y = Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -32%

Clip X = 0°

Clip Y = 0°

3D Image Processing - Panel B

3D Volume Projection = Precise

Appearance = Threshold 2% all channels, Ramp 98% 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 presents a three-dimensional volume projection of Rabbit anti-Human BAP1.p Polyclonal IgG. It is counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red) and Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green), along with DAPI (blue), using super-resolution Airyscan imaging. The image features a cut plane on the X-Y axis (blue outline), positioned at -32 degrees, with angles X and Y set to 0 degrees (flat) to enhance the visualization of BAP1.p within the nucleus A region of interest (ROI) is indicated by a white box in Panel A for further investigation in Panels B and C.

Panel B - This panel displays a split view from the ROI in Panel A, at a z-plane 81 out of 161, 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), while the lower right section features a merged image of the three channels.

Panel C - This panel provides a three-dimensional volume projection of Rabbit anti-Human BAP1.p Polyclonal IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red) only so that we may further investigate what super resolution provides in terms of 3-dimensional localization and structure.

Identifyn® SRM Alexa Fluor™ 647 has been pseudo-colored from its default color of red to white to enhance visual contrast for examination.

Note - At Airyscan resolution, the "pan-nuclear" or diffuse nature of BAP1.p observed in diffraction-limited applications, such as widefield, widefield SIM, and confocal microscopy, is enhanced with new structural details. s seen in studies of nuclear proteins

through Identifyn's™ step-down method, what was previously perceived as ubiquitous or pan-nuclear now appears interconnected and organized.

Image Corrections

None

Image by J.K. Bennett

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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