

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human BAP1 Polyclonal***Cat ID# - PA-RP 000012*Identifyn® Secondary Antibody**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057*

BAP1 (BRCA1-associated protein 1) is a deubiquitinase with critical roles in DNA repair, chromatin dynamics, and cell cycle regulation. Its ability to interact with other proteins central to the DNA damage response is significant in maintaining genomic stability. BAP1 has several identified protein-protein interactions within the HR pathway, which are critical to DNA Damage Response (DDR) signaling.

BAP1 and BRCA1, BARD1, Rad51 and Others

BRCA1 is a tumor suppressor gene critical for homologous recombination (HR), a high-fidelity DNA repair pathway for double-strand breaks. BAP1's interaction with BRCA1 and other DNA repair proteins, such as BARD1 and Rad51, is linked to its deubiquitinating activity, suggesting that it regulates critical protein function during DNA repair. This relationship may influence BRCA1's stability, recruitment to DNA damage sites, or ability to mediate HR. This interaction underlines the importance of BAP1 in promoting accurate DNA repair and maintaining genomic stability.

BAP1 and ATM

ATM (ataxia-telangiectasia mutated) is a central kinase in the DNA damage response, primarily activated by DNA double-strand breaks. It phosphorylates various downstream targets to initiate DNA repair pathways, including homologous recombination and non-homologous end joining (NHEJ). While the direct interaction between BAP1 and ATM isn't as well-documented as other interactions, BAP1 may indirectly influence ATM's activity by affecting the stability or function of proteins involved in DDR. Deubiquitination (BAP1) and phosphorylation (ATM) may well affect how cells respond to DNA damage.

BAP1 and HERC2

HERC2 is an E3 ubiquitin ligase involved in regulating protein ubiquitination. It has a known role in genomic stability and is implicated in the DNA damage response. BAP1's interaction with HERC2 likely involves deubiquitination, suggesting that BAP1 could regulate HERC2's activity or modulate its effects on other proteins in the DNA repair pathways. This interaction could be critical in balancing ubiquitination and deubiquitination, impacting DNA repair processes and cell cycle control.

ASXL1/ASXL2

ASXL1 and ASXL2 (additional sex combs-like 1 and 2) are other key interactors of BAP1. This complex can influence chromatin remodeling, potentially affecting the accessibility of DNA repair proteins to sites of DNA damage.

Implications in DNA Repair

These interactions underscore BAP1's involvement in DNA repair pathways and the broader DNA damage response. By interacting with BRCA1, BAP1 may facilitate

homologous recombination, while interactions with ATM and HERC2 highlight its role in broader DDR signaling. Critical interaction with ASXL1/ASXL2 highlights its importance in chromatin remodeling, potentially affecting the accessibility of DNA repair proteins to sites of DNA damage. BAP1's deubiquitinating activity can stabilize essential proteins, promote the proper assembly of repair complexes, and maintain chromatin structure, all of which are necessary for efficient DNA repair and genomic integrity. Mutations in BAP1 that disrupt these interactions can increase genomic instability and are associated with various cancers, underscoring the clinical relevance of these protein interactions for understanding disease mechanisms and developing therapeutic strategies.

Method

HeLa cells were grown in a μ -Slide 8 Well high-Glass Bottom [Ibidi chamber](#). 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, Rabbit anti-Human BAP1 Polyclonal was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3 times in Identifyn®'s proprietary Wash Buffer, and Identifyn®'s 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:200. Cells were washed 5 times in Identifyn®'s proprietary Wash Buffer followed by the addition of Identifyn®'s proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 Silicon oil Objective, was used with the following parameters:

Calibration -

Calibration follows Identifyn®'s Calibration Standard Operating Procedure, which can be found here.

<https://identifyn.com/storm-calibration>

Image Acquisition -

SRX Software - Under the 'Single Molecule Localization' tab, **'Record'** is selected and contains the following workflow tabs and parameters.

Configuration Tab - Capture Configuration

Number of Probes: 1

Number of Simultaneous Probes: 1

Number of Reference Probes: 1

Camera: Both Cameras

Color Channel: Both Channels

Probe Capture Mode: Interleaved

Z -Stack Capture Mode: Interleaved

Multiple Location Capture: Not selected

Post Record Reference: Not Selected

Fluidics: Not Selected

Autofocus Drift Correction: On

Microscope Configuration

Biplane Module: 635 nm Dichroic

Objective: Silicon Selected

Stage Insert: Rectangular Selected

Course Focus Position: 8-Well Chamber Selected

Dichroic: SuperRes

PSF: (Red, Orange)

Heater: Current: 26

Camera Configuration

Recording Vertical Field of View: 100%

Widefield Camera Exposure Time: 100 ms
Super Resolution Camera Exposure Time: 20 ms
Widefield Camera Binning: 1
Per-Probe Exposure Time: Selected

Probe Configuration

Predefined Probe: First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)

Photoactivation/Imaging Laser: 0% Selected
Exposure Time: 20 ms Selected

Experiment Configuration

Record Workflow: Selected Options
View Mode: Widefield 200 µm
Capture Mode: Live

Stage and Piezo Selection**Examination of the Sample**

Cell(s) or Cellular Structure Localization: The red-bordered box identifies the area of the SuperRes view and defines the appropriate cell(s) or cellular structure. The cell(s) or structures are optimally focused.

Piezo Current: 182.6 µm

Probe 1 Laser control 640 nm (635 nm Dichroic)

Widefield 640 nm: 0.1X Neutral Density Filter: Selected, @ 0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100 ms
Default values are maintained unless otherwise noted.
Widefield Laser: "Widefield 640 nm" was deselected, and "calibrate" under Autofocus was selected.

Autofocus - Calibration Values

Current Position: -0.979
Current Intensity: 4.5
Calibration Value: -5070 nm/unit
Autofocus Mode: Pre-Capture

View Mode

SuperRes: 50 µm is selected

Capture Mode

Live

Stage and Piezo Selection

Cell(s) or Cellular Structure Localization - Positioning of the target Cell or Subsection of the Target Cell is optimized and optimally focused.

Piezo Record: Begin: 182.6; End: 183.8

Probe 1 Laser control 640 nm (635 nm Dichroic)

SuperRes 640 nm: 0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input

Reference Probe 1 Laser control 640 nm (635 nm Dichroic)

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20 ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200

Z Positions: 6

Cycles: 25

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635 nm Dichroic)

Laser Power: 30

Expert Configuration Options - The Localization Tab

Beyond the values shown, all other parameters were set to default values.

Localization

Cutout Width: 16 px

Max Frame Accumulation: 1 frame

Max Accumulation Offset: 1 px

Fiducial at Max Accumulation: Not selected

Max Particles Per Frame: No Max

Background SLPE Removal: Not Selected

Cutout/Background Removal: Not Selected

Preview Cutout Positions: Selected

Record Workflow - Localization Tab**Data Collection**

The folder is created automatically with the localization file for this image acquisition.

Recording Summary

All parameters were carried over from previous tabs and are kept except:

Probe1: BG threshold: 15

Region of Interest -

Not selected

Display -

Was deployed to inspect if the BG threshold applied chooses localizations in an expected manner

Record Workflow - View Tab**Region of Interest**

Default values

Visualization Parameters

Point Splatting

Sizing Mode: Constant

Particle size: 30 nm

Color by: Depth

Color Table: Single

Opacity Mode: Depth; Opacity 1: 0.40

Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)

Time Intervals: 8 Intervals

Pixel Size: 50 nm

Smoothing: 8.00

Advanced Particle Filters - Applied to the drift-corrected data with the following parameters

Radial precision: 30

Axial precision: 70

The other parameters were not applied.

Statistical Analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.1 μm

Maximum Particle Count to Form Cluster: 10

Compute Hulls: Not Selected

Compute Density Isosurfaces: Not Selected

Edit Settings: All Probes

Compute: Selected

Following Cluster Analysis Computation, under the Results Tab, ID Unassigned:

Deselected

Image by P. Raut

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