

**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')<sub>2</sub> (H + L)***Cat ID# - SA 000063*

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  $\mu$ -Slide 8 Well high Glass Bottom [Ibidi chamber](#). DNA damage was induced via treatment with 2 $\mu$ M Etoposide-spiked complete growth media for 6 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, was incubated for 1 hour at room temperature at a dilution of 1:200. Cells were washed 3X in Identifyn's™ proprietary Wash Buffer, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:200. Cells were washed 5X 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: 635nm 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: 100ms  
Super Resolution Camera Exposure Time: 20ms  
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: 20ms Selected

### Experiment Configuration

Record Workflow: Selected Options  
View Mode: Widefield 200µm  
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 a suitable cell(s) or cellular structure. The cell(s) or structures are optimally focused.  
Piezo Current: 179 µm

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

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

### Advanced Tools Option

Widefield camera Exposure Time: 100ms  
Default values are maintained unless otherwise noted.  
Widefield Laser: "Widefield 640nm" 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: 50µm 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: 175; End: 177.2

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

SuperRes 640nm: 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: 20ms  
Default values are maintained unless otherwise noted.

### Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200  
Z Positions: 11 using Steps: 0.2µm (200nm)  
Cycles: 15  
Time Points: 1  
Pause Between Cycles: Not Selected  
Automatic Photoactivation Laser Control: Not Selected

### Probe 1 Laser control (640 nm (640 nm Dichroic)

Laser Power: 70%

### Expert Configuration Options - The Localization Tab

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

### Localization

Cutout Width: 16px  
Max Frame Accumulation: 1 frame  
Max Accumulation Offset: 1px  
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: 20nm

Color by: Constant

Color Table: Single

Opacity Mode: Depth; Opacity 1: 0.4

Front-to-back Attenuation: Not Selected

## Post Acquisition analysis - View Tab

**Drift Correction** - Computed with the following parameters:

Method: Particle (direct)

Time Intervals: 10 Intervals

Pixel Size: 50nm

Smoothing: 8.00

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

PSF Z offset: -600 to +600

Radial precision: 25

Axial precision: 65

The other parameters were not applied.

## Statistical analysis - NA

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Image by P. Raut

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