Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human Ku70 Polyclonal, IgG
Cat ID# - *PA-RP 000006*

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')$_2$ (H + L)
Cat ID# - SA 000063

Ku70 is a crucial protein involved in the Non-Homologous End Joining (NHEJ) pathway, one of DNA's central mechanisms for repairing double-strand breaks (DSBs).

Recognition and Binding to DNA Breaks: Ku70 and Ku80 form the Ku heterodimer. This heterodimer has a high affinity for DNA ends and is responsible for recognizing and binding to DSBs. Its structure allows it to slide onto the DNA strand and form a ring around the break, stabilizing it.

Recruitment of Additional NHEJ Proteins: After binding to the DNA break, the Ku heterodimer acts as a scaffold, recruiting and assembling other proteins essential for the NHEJ pathway. This includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which are crucial in further processing the break.

Protection of DNA Ends: By binding to the ends of the DNA at the site of the break, Ku70/80 helps protect the DNA from further degradation or unwanted processing, ensuring that the ends are preserved for the repair process.

Alignment of DNA Ends: Ku70/80 helps align the broken DNA ends, facilitating the ligation step in NHEJ. This alignment is crucial for accurate repair, even though NHEJ does not require extensive sequence homology.

Participation in End Processing and Ligation: Once other NHEJ factors have been recruited, Ku70/80 can assist in processing DNA ends if necessary. Ultimately, it helps bring the DNA ends together for ligation by the ligase complex (such as DNA Ligase IV, XRCC4, and XLF), completing the repair process.

In summary, Ku70, as part of the Ku heterodimer, plays a central role in the NHEJ pathway by recognizing and binding to DNA breaks, recruiting and organizing other repair proteins, protecting DNA ends, and facilitating the alignment and ligation necessary to repair double-strand breaks.

Method

HeLa cells were grown on µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2µM 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, Rabbit anti-Human Ku70 Polyclonal, was incubated for 1 hour at room temperature at a dilution of 1:100. 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 1:250 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.

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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

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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 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: 177 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

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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: 174; End: 175.2

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: 6 using Steps: 0.2µm (200nm)
Cycles: 25
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: 90%

Expert Configuration Options - The Localization Tab

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

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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 - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

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Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.1um (900nm)
Maximum Particle Count to Form Cluster: 10
Computer 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

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Image by P. Raut

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