

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human Ku70 Polyclonal, IgG

Cat ID# - PA-RP 000006

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 Donkey Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000042

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 acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM 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 Ku70 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 Donkey Anti-Mouse, F(ab')₂ (H + L) 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 = 201 Slices (6 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1066 X 1066
Image Size (Scaled) = 62.72 µm X 62.72 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.41 mm, Y: 51.38 mm
Stage Position = X: 84.41 mm, Y: 51.38 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-plane 85 of 201 total - Panel B

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

Z-Stack = 201 Slices (6 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1038 X 650
Image Size (Scaled) = 61.08 µm X 38.25 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.41 mm, Y: 51.38 mm
Stage Position = X: 84.41 mm, Y: 51.38 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 65 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 639 nm @0.06%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1

Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 636-732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.9 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 50 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 488 nm @0.04%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 489-550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.8 (3D, Auto)

DAPI -

Reflector = None

Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 μ m
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 μ s
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430-499
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.3 (3D, Auto)

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping Enabled
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 = A clipping plane was introduced to pronounce regions of special interest.

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

Position of Clip = -30%
Clip X = 0°
Clip Y = 0°

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

Position of Clip = -51%
Clip X = 30°

Clip Z = 0°

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

Position of Clip = 0%

Clip Y = -30°

Clip Z = 0°

3D Image Processing - Panel C

3D Maximum Projection = Precise

Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Tone Mapping Enabled

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 = A clipping plane was introduced to pronounce regions of special interest.

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

Position of Clip = -13%

Clip X = 30°

Clip Z = 0°

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

Position of Clip = 0%

Clip Y = -30°

Clip Z = 0°

Summary

Panel A - This panel presents a 3-dimensional Maximum Projection to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn® SRM Alexa Fluor™ 647 (white, changed from default red in Zeiss Zen software to enhance visual contrast) and an Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI (blue), at confocal resolution. The image employs a triple cut plane on the X-Y (blue outline), the X-Z (green outline), and the Y-Z planes (dark red outline). The X-Y cut plane uses a Position of -30%, and the angles X and Y are set to zero, or flat. In the X-Z cut plane, the Position is - 51%, the angle X is at 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is 0%, the angle X is at -30 degrees, and the angle Z is 0 degrees. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment.

Panel A includes an inset of the same image at z-plane 85 of 201 (lower right corner) showing the Region of Interest (ROI) to be further examined in panels B and C.

Panel B – This panel presents a 2-dimensional Image of the ROI shown in Panel A. The z-plane 85 was used to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG, labeled with Identifyn[®] SRM Alexa Fluor[™] 647 (red), surrounded by Alpha Tubulin primary antibody with Identifyn[®] SRM Alexa Fluor[™] 488 (green). DAPI was intentionally omitted to provide better visualization of the Ku70 nuclear signal.

Panel C - This panel presents a 3-dimensional Maximum Projection of the ROI shown in Panel A to demonstrate further the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn[®] SRM Alexa Fluor[™] 647 (red) and an Alpha Tubulin primary antibody with Identifyn[®] SRM Alexa Fluor[™] 488 (green), at confocal resolution employing Zeiss Zen LSM Plus (LSMP). This image utilizes a double cut plane, or wedge, on the X-Z (green outline) and Y-Z planes (dark red outline). The X-Z cut plane, the Position is -13%, the angle X is at 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is 0%, the angle X is at -30 degrees, and the angle Z is 0 degrees. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment very nicely, as it is organized and surrounded by the cytoplasmic Alpha Tubulin.

Image Corrections

None

Image by J.K. Bennett

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Data Presentation by P.D. Amistoso

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