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 PB. 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.

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The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), 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 Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 878 X 929
Image Size (Scaled) = 96.16 µm X 101.75 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03 µm
Binning Mode = 2,2

488 -

Reflector = 32 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4

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Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - *Panel A*

The top-left panel features Rabbit anti-Human Ku70 Polyclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, $F(ab')_2$ (H + L); the top-right panel features nuclear pore complexes visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, $F(ab')_2$ (H + L); the bottom-left panel features DAPI nuclear staining. The bottom-right panel features the merged image of all channels.

Profile View Display - *Panel B*

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0 and Max 0), Y (Min 0 and Max 1)

Summary

Panel A - Split View illustrates the signal and localization of the Rabbit anti-Human Ku70 Polyclonal, IgG antibody. The upper left section features the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn™SRM Alexa Fluor™ 647 (red). The upper right image shows the Nuclear Pore Complex (NPC) with Identifyn® SRM Alexa Fluor™ 488 (green), while the lower left image is a DAPI nuclear staining (blue). The lower right presents a merged image of the three channels. The merge demonstrates that Ku70 is strongly localized to the nucleus, as indicated by DAPI staining, and the NPC staining, which marks the nuclear periphery, confirms its presence within the nuclear compartment following Etoposide-induced DNA damage.

Panel B - Displays a colocalization view with DAPI (Y-axis) and the Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn® SRM Alexa Fluor™ 647 (X-axis). The colocalization measurement encompasses the entire image. This measurement demonstrates that Ku70 is strongly colocalized with DAPI, suggesting that, as expected for this protein, it is located within the nuclear compartment.

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As widefield is the first in our series of the "step down" method, as expected and seen as highly consistent, the staining appears pan-nuclear. As we move through widefield SIM and Confocal, much will stay the same; however, as we cross into super resolution, we will see that Ku70 displays 3-dimensional localization and structural details.

Image Correction

None

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Image Analysis by B.T. Bennett
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