

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, [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 Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 16 of 31 total - Panels A & C

Z Stack - (3D)

Z-Stack = 31 Slices (3 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 978 X 971
Image Size (Scaled) = 139.71 µm X 138.71 µm
Bit Depth = 14 Bit
Image Center Position = X: 81.11 mm, Y: 50.59 mm
Stage Position = X: 81.11 mm, Y: 50.59 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - Panel D

Z-Stack = 31 Slices (3 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 289 X 316
Image Size (Scaled) = 41.29 µm X 45.14 µm
Bit Depth = 14 Bit
Image Center Position = X: 81.11 mm, Y: 50.59 mm
Stage Position = X: 81.11 mm, Y: 50.59 mm
ROI Center Offset = X: 1.14 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 = 1.25
 Imaging Device = 807 mono
 Camera Adapter = 1X
 Depth of Focus = 1.30 μm
 Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @16.00%
 Exposure Time = 150 ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.25
 Imaging Device = 807 mono
 Camera Adapter = 1X
 Depth of Focus = 1.00 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 @6.00%
 Exposure Time = 12 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.25
 Imaging Device = 807 mono
 Camera Adapter = 1X
 Depth of Focus = 0.90 μm
 Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mod" "Optical Sectioning" - enabled correction selected.

Normalization, Phase Corrector, Fourier Filter, and Deconvolution **were NOT used.**

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. This image was taken at Z-Position 16 of 31.

Profile View Display - Panel B & C

Z-Position = 16 of 31

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 104.6), Y (Min 0 and Max 3414)

3D Image Processing - Panel A

3D Maximum Projection = Precise

Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Enabled Tone Mapping

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 = Clipping planes are 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 = 0%

Clip X = 0°

Clip Y = 0°

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 NPC staining, which marks the nuclear periphery, confirming its presence within the nucleus following Etoposide-induced DNA damage.

Panel B - This panel utilizes a line profile display to demonstrate localization and fluorescent intensity. The X-axis represents distance, while the Y-axis indicates intensity. The data reveal that the signals for the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red) and Nuclear Pore Complex (NPC) with Identifyn® SRM Alexa Fluor™ 488 (green) occupy the same location along the X-axis (the nuclear compartment). This finding confirms that the Rabbit anti-Human Ku70 Polyclonal, IgG, and NPC are localized on the Z-plane at 16 of 31, indicating they occupy the same spatial area in the image, as noted by the NPC signal. The Y-axis also presents the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red), which exhibits vigorous fluorescent intensity following Etoposide-induced DNA damage.

Panel C - This panel displays z-plane 16 for both the BRCA1/647 and NPC/488 channels, with a line drawn across two nuclei to illustrate the source of our measurements in Panel B.

Panel D - This panel presents a 3-dimensional Maximum Projection from the ROI in Panel A to demonstrate further the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG. The projection shows Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red), and Nuclear Pore Complex (NPC) with Identifyn® SRM Alexa Fluor™ 488 (green), at widefield SIM resolution. The image employs an X-Y cut plane (outlined in blue. The position is 0%, and the angles X and Y are both at 0 degrees (flat). The cut plane is used to reiterate the NPC designating the nuclear compartment periphery and the Ku70 nuclear localization.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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