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

Plus Damage – *Panel A*

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® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

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No Damage – *Panel B*

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527. 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® 647 secondary antibody 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:

Z-Stack – (3D) – *Panel A*

Z-Stack = 31 z-planes (3 µm) – *Uses Z-plane 16 for Analysis.*

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm x 100µm
Image size (Pixels) = 978 X 971
Image Size (Scaled) = 139.71µm X 138.71µm
Bit Depth = 14 Bit
Image Center Position = X: 81.11µm, Y: 50.59µm
Stage Position = X: 81.11µm, Y: 50.59µm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

Z-Stack – (3D) – *Panel B*

Z-Stack = 31 z-planes (3 µm) – *Uses Z-plane 16 for Analysis.*

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm x 100µm
Image size (Pixels) = 707 X 580
Image Size (Scaled) = 101.00µm X 82.86µm
Bit Depth = 14 Bit
Image Center Position = X: 74.56µm, Y: 49.88µm
Stage Position = X: 74.56µm, Y: 49.88µm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

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

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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

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

Profile View Display - *Panel A*

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 95.9), Y (Min 0 and Max 4948)

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 0.0 and Max 86.7), Y (Min 1 and Max 1816)

Summary

Panel A – DNA damage was induced via treatment with 2µM Etoposide–spiked complete growth media for 24 hours. This panel presents a profile view of z-plane 16 of 31, showing the optimal signal (center z-position for the nucleus) for the Rabbit anti-Human Ku70 Polyclonal, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red). On the Left is the histogram; the x-axis represents distance, and the y-axis represents fluorescent intensity. ***The Sample Mean (average intensity/distance) is equal to 2030.556 Fluorescent Arbitrary Units (A.U.)*** across the line shown in the right-hand image (575 data points), where the measurement was taken.

Panel B – DNA damage was **NOT** induced. This panel presents a profile view of z-plane 16 of 31, showing the optimal signal (center z-position for the nucleus) for the Rabbit anti-Human Ku70 Polyclonal, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red). On the Left is the histogram; the x-axis represents distance, and the y-axis represents fluorescent intensity. ***The Sample Mean (average***

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intensity/distance) is equal to 1106.503 Fluorescent Arbitrary Units (A.U.) across the line shown in the right-hand image (589 data points), where the measurement was taken.

Panel C – This panel displays the ***acquisition settings used for both images***, showing that they are identical, with the same excitation input from the X-Cite Xylis Lamp at 10.00% and exposure times of 100 ms.

Summary - The Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody (native or non-modified protein) displays an increase in total protein as a measurement of fluorescence While the Sample Mean for the etoposide-treated cells is ~50% higher in signal, one could argue that it may represent an increase in Ku70 protein production in response to the damage; however, we make no biological claims regarding function.

Image Corrections

None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

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