

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N)***Cat ID# - PA-RR-000008*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000015*

ATM (ataxia-telangiectasia mutated) plays a crucial role in the homologous recombination (HR) pathway, an essential mechanism in the repair of DNA double-strand breaks (DSBs).

ATM is a serine/threonine protein kinase activated in response to DSBs. Once activated, ATM phosphorylates numerous downstream targets, including proteins in DNA repair pathways such as HR. In the context of HR, ATM promotes the repair process by phosphorylating key proteins involved in DNA repair, such as BRCA1 (Breast Cancer 1) and RAD51.

BRCA1 is known to facilitate RAD51 loading onto single-stranded DNA at DSB sites, a crucial step in the HR pathway. ATM-mediated phosphorylation of BRCA1 facilitates the recruitment and stabilization of RAD51 at DNA damage sites, thereby enhancing the HR repair process. ATM also plays a critical role in cell cycle checkpoint control, leading to the initiation of cell cycle checkpoints, which temporarily halt cell cycle progression to allow time for DNA repair. Additionally, ATM activation can trigger the apoptotic cell death pathway, eliminating severely damaged DNA and reducing the propagation of genetic abnormalities.

Overall, ATM serves as a critical guardian of genome integrity, ensuring the maintenance of genomic stability and preventing the accumulation of mutations that could lead to various diseases, including cancer.

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 ATM Recombinant Monoclonal, IgG- (BLR021N), was incubated for 1 hour at room temperature at a 1:250 dilution. 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](#).

The second primary antibody, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (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-plane 72 of 142)

Z-Stack = 142 Slices (1.41µm)

Channels = 3
Scaling (Per Pixel) = 35.30nm X 35.30nm x 30.00nm
Image size (Pixels) = 1248 X 1248
Image Size (Scaled) = 44.05 µm X 44.05µm
Bit Depth = 16 Bit
Image Center Position = X: 88.14 mm, Y: 51.41 mm
Stage Position = X: 88.14 mm, Y: 51.41 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel B

Z-Stack = 142 Slices (1.41µm)

Channels = 3
Scaling (Per Pixel) = 35.30nm X 35.30nm x 30.00nm
Image size (Pixels) = 639 X 756
Image Size (Scaled) = 22.55 µm X 26.68µm
Bit Depth = 16 Bit
Image Center Position = X: 88.14 mm, Y: 51.41 mm
Stage Position = X: 88.14 mm, Y: 51.41 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 5.00 AU / 328µm
LMS MBS = MBS 488/639 MBS 405/730
LMS SBS = SBS SP 640
Laser Wavelength = 639nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82µs
Frame Time = 6.25s
LSM Scan Speed = 7
Scan Direction = Bidirectional

Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 5.00 AU / 250µm
LMS MBS = MBS 488/639 MBS 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488nm @0.04%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82µs
Frame Time = 6.25s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution 9.9 (3D, Auto)

DAPI -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 5.00 AU / 214µm
LMS MBS = MBS 488/561 MBS 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled

Scan Mode = Frame
Scan Zoom = 2.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82µs
Frame Time = 6.25s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution 7.6 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode "SR"
Super Resolution Auto Filter Selected
Process 3D Current Image
Created

Split View - Panel A

The upper left features Rabbit anti-Human ATM Recombinant Monoclonal, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the upper right panel shows Alpha Tubulin monoclonal primary antibody counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L). The lower left panel features DAPI nuclear staining. The lower right panel features the merged image of all channels. This split View was taken at Z-Position 72 of 142.

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel B

The Clipping plane is introduced to demonstrate the specificity of the Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N) to the nuclear compartment.

X-Y = Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and ATM signal. A red outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.

Position of Clip = 20%

Clip Y = -0°

Clip Z = 0°

Image Corrections

None

Image by J.K. Bennett

Image Analysis and Data Presentation B.T. Bennett

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