

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 the loading of RAD51 onto single-stranded DNA at the site of DSBs, which is 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 Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A, Total Image Acquisition

Z-Stack = 278 Slices (8.31µm)

Channels = 3
 Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
 Image Size (Pixels) = 5056 X 5056
 Image Size (Scaled) = 106.75µm X 106.75µm
 Bit Depth = 16 Bit
 Image Center Position = X: 58.60mm, Y: 36.42mm
 Stage Position = X: 58.60mm, Y: 36.42mm
 ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panels B & C, Region of Interest

Z-Stack = 184 Slices (5.49µm) - The Gallery tool was used to reduce the z-stack to provide clarity.

Channels = 2
 Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
 Image Size (Pixels) = 1486 X 1775
 Image Size (Scaled) = 31.37µm X 37.47µm
 Bit Depth = 16 Bit
 Image Center Position = X: 58.60mm, Y: 36.42mm
 Stage Position = X: 58.60mm, Y: 36.42mm
 ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 640
 Channel Name = T1-Axiocam 820 -SR
 Excitation Wavelength = 640
 Emission Wavelength = 729
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 120ms

Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @2.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 27.5µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @1.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 27.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @1.5%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D

Result Sampling = 4

Channels = 3

Algorithm = SIM

SIM Settings = Standard

Auto Sharpness = On

Sharpness = 9.6715 (647), 11.5549 (488), 9.7630 (DAPI)

Fitting = Fast Fit

Normalization = Automatic

3D Image Processing - Panel A

3D Maximum Projection = Precise

Appearance = Threshold 2% (647), Threshold 2% (488), Threshold 2% DAPI, 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)

3D Image Processing - Panel B

3D Maximum Projection = Precise

Appearance = Threshold 3% (647), Threshold 4% DAPI, 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)

3D Image Processing - Panel C

3D Volume Projection = Precise

Appearance = Threshold 2% (647), Ramp 98%, Maximum 100%

Background = Black

Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, 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)

Image Corrections

None

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