

Identifyfyn Standards for Optical Microscopy Methods™Identifyfyn® Primary Antibody**Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N)***Cat ID# - PA-RR-000008*Identifyfyn® Secondary Antibodies**Identifyfyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057***Identifyfyn® 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. Identifyfyn® 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. Identifyfyn® 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) - Panels A & B (Z-plane 68 of 141)

Z-Stack = 141 Slices (4.20µm)

Channels = 3

Scaling (Per Pixel) = 0.059µm X 0.059µm x 0.030µm

Image size (Pixels) = 1017 X 1017

Image Size (Scaled) = 59.81 µm X 59.81 µm

Bit Depth = 16 Bit

Image Center Position = X: 88.54 mm, Y: 47.92 mm

Stage Position = X: 88.54 mm, Y: 47.92 mm

ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

Reflector = None

Contrast Method - Fluorescence

Pinhole = 1.00 AU / 66µm

LMS MBS = MBS 488/639 MBS 405/730

LMS SBS = SBS SP 615

Laser Wavelength = 639nm @0.3%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 2.2

Rotation = 0°

Sampling = 1.20

Pixel Time = 0.33µs

Frame Time = 3.36s

LSM Scan Speed = 12

Scan Direction = Bidirectional

Line Step = 1

Averaging = 8

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.4

Detection Wavelength = 636 - 732

Imaging Device = GaAsP-PMT3

Detector Type = GaAsP-PMT

Detector Gain = 500 V

Detector Offset = 0

Detector Digital Gain = 1.0

LSM Plus Mode = Filter: 7.1 (3D, Auto)

488 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 50µm
LMS MBS = MBS 488/639 MBS 405/730
LMS SBS = Mirror
Laser Wavelength = 488nm @0.1%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 489 - 517
Imaging Device = GaAsP-PMT3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.2 (3D, Auto)

DAPI -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 43µm
LMS MBS = MBS 488/561 MBS 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4

Detection Wavelength = 430 - 470
Imaging Device = GaAsP-PMT3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.8 (3D, Auto)

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 68 of 141.

Profile View Display - Panel B, (Histogram) Panel C (Image Measured)

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 65.1), Y (Min 13 and Max 25263)

The average fluorescent intensity across the two nuclei measured was **9671.953**, deploying the **639nm laser** at an **input power of 0.3%**

Zeiss Zen Settings - Panel D, Image Acquisition Settings

A clipped image from Zeiss Zen image acquisition settings for the Rabbit anti-Human ATM Recombinant Monoclonal, IgG (BLR021N) image acquisition, showing all three channels acquired for transparency.

Image Corrections

None

Image by J.K. Bennett
Image Analysis and Data Presentation B.T. Bennett

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