

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 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 (Maximum projection) and Panel B (Colocalization, z-plane 43 of 81)

Z-Stack = 81 Slices (8.0 µm)

Channels = 3

Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm

Image Size (Pixels) = 1147 X 957

Image Size (Scaled) = 163.86 µm X 136.71 µm

Bit Depth = 14 Bit

Image Center Position = X: 76.72 mm, Y: 50.18 mm

ROI Center Offset = X: 76.72 µm, Y: 50.18 µm

647 -

Reflector = 50 Cy5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @ 15%

Exposure Time = 100ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

Camera Adapter = 1X

Depth of Focus = 1.30µm

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 @ 20.00%

Exposure Time = 200 ms

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.25

Imaging Device = Axiocam 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 @ 8.0%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90 μm
Binning Mode = 2,2

Post Processing - SIM Processing

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

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

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 - Panel A

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 = 9%
Clip Y = -0°
Clip Z = 0°

Colocalization View - Panels B and C

Horizontal Axis -DAPI Threshold 32, Range Auto
Vertical Axis - ATM/647, Threshold 70, Range Auto

Colocalization data show that both Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N) and the DAPI nuclear staining are strongly colocalized, with both residing within the nuclear compartment following DNA damage.

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, as well as the two other channels collected, 488 and DAPI. We demonstrate that an X-Cite LED light input of 10% and a camera exposure time of 100 ms were sufficient to capture the image, further demonstrating excellent localization and high brightness for the Identifyn® Rabbit Recombinant Monoclonal ATM, combined with the Identifyn® 647 secondary antibody.

We also note that the Identifyn® 488 secondary required a 15% LED input and a 150ms exposure.

Image Correction

None

Image by E.F. Simon
Image Analysis and Data Presentation by B.T. Bennett

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