

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

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](#).

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam 705 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D) - Panel A & B

Channels = 2
Scaling (Per Pixel) = 0.110 μm X 0.110 μm
Image size (Pixels) = 472 X 487
Image Size (Scaled) = 51.70 μm X 53.34 μm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 μm , Y: 0.00 μm
ROI Center Offset = X: 0.00 μm , Y: 0.00 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 = 150ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 1.03 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 @ 10%
Exposure Time = 20ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 0.72 μm
Binning Mode = 2,2

Split View - Panel A

The left panel features Rabbit anti-Human ATM Recombinant Monoclonal, IgG (BLR021N), the middle panel shows DAPI nuclear staining, and the right panel displays the merged image of all channels. The Image demonstrates the nuclear localization and brightness of the ATM Rabbit Recombinant antibody.

Profile View Display - Panel B

Profile Definition = Stroke Thickness 105, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 50.5), Y (Min 43 and Max 1458)

The profile display shows the ATM signal across two nuclei, highlighting specificity to the nucleus and relative fluorescent Intensity.

Zeiss Zen Settings - Panel C, Image Acquisition Settings

A clipped image from Zeiss Zen image acquisition settings for the Rabbit anti-Human ATM Recombinant Monoclonal, IgG (BLR021N) image acquisition. We demonstrate that an X-Cite LED light input of 15% and a camera exposure time of 150 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.

Image Correction - Panel B

Identifyn® ATM/647 staining was changed from its default red in Zeiss Zen to white to enhance this visualization.

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

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