

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, F(ab')₂ (H + L)

Cat ID# - SA 000063

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 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:

2- Dimension Image - Panels A & B (note settings and image sizes were matched for the control to the data from the confocal within this data set)

Channels = 3
Scaling (Per Pixel) = 0.059 μ m X 0.059 μ m
Image size (Pixels) = 1017 X 1017
Image Size (Scaled) = 59.81 μ m X 59.81 μ m
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: 0.00 μ m, Y: 0.00 μ m

647 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 66 μ m
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 = 0.33 μ s
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)

Profile View Display - Panel A

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%**

Profile View Display - Panel B

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 42.9), Y (Min 4 and Max 3092)

The average fluorescent intensity across the two nuclei measured was 821.0316, deploying the **639nm laser** at an **input power of 0.3%**

Summary -

We note that when using the same settings in the presence of damage (panel A) and in the absence of induced DNA damage, we observe a ~10-fold decrease in the ATM signal. We also note this is not a modified version of ATM, and in such the increase in total volume of protein as measured by the fluorescent intensity, reveals the activation and recruitment of ATM in response to the damage, but does not explicitly show the later version of the ATM response which would be foci aggregates at breaks sites, as this antibody recognizes the active form. An increase in total protein is expected;

Image Corrections

None

Image by J.K. Bennett

Image Analysis and Data Presentation B.T. Bennett

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