

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody.

Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N)

Cat ID# - PA-RR 000008

Expected Molecular Weight: 350 kDa

HeLa cells were damaged with 200 µM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with [NuPAGE™ LDS Sample Buffer \(4X\)](#), heated at 80 °C for 10 min, and subjected to electrophoresis using [NuPAGE™ Tris-Acetate Mini Protein Gels, 3 to 8%, 1.0 mm, 2D-well](#) in a [Mini Gel Tank](#). Thermo Fisher's HiMark [Prestained Protein Standard](#) was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the [iBlot™ 3 Starter Kit, PVDF](#). The transfer membrane was blocked with [Blocker™ Casein](#) for 1 hour, then washed 5 times with PBS. The blocked membrane was incubated with Rabbit anti-Human ATM Recombinant Monoclonal, IgG for 2 hours, followed by five washes in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5 TIMES with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = [Azure Sapphire FL](#) with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane(s)

Scan Settings

Detection mode = Fluorescence

Laser = 488nm

Emission Filter = 513±17nm

Detector = PMT

Intensity = 2

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: K. Small

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