

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Mouse anti-Human, ATRIP Monoclonal, IgG2b - (4H6H4/G3)***Cat ID# - PA-MM 000003**Expected MW: 82 kDa*

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and RIPA lysis buffer, 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™ 4 to 12% Bis-Tris, 1.0–1.5 mm, Mini Protein Gels](#) in a [Mini Gel Tank](#). The Thermo Fisher's [PageRuler™ Prestained Protein Ladder, 10 to 180 kDa](#), 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 Mouse anti-Human, ATRIP Monoclonal, IgG for 2 hours, followed by five washes in 1X PBS. Identifyn™ SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour and washed 5X 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 = 685nm
Emission Filter = 720±24nm
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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