

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human ATR.p (Threonine 1989) Recombinant Monoclonal, IgG - (BLR032P)***Cat ID# - 000013**Expected MW: 301 kDa*

HeLa cells were treated with 2 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a nuclear 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 on [NuPAGE™ Tris-Acetate Mini Protein Gels \(3-8%, 1.0 mm\)](#) in a [Mini Gel Tank](#). The Thermo Fisher™ [HiMark™ Pre-stained 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 ATR.p (Threonine 1989) Recombinant Monoclonal, IgG - (BLR032P) for 20 hours, followed by 5 washes in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5 times with 1X 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 Imaging.

Scan Settings

Detection mode = Fluorescence

Laser = 638 nm

Emission Filter = 676 ± 37 nm

Detector = PMT

Intensity = L3

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

NONE

Image: S. Khanal

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