

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human APE1 Polyclonal, IgG***Cat ID# - PA-RP 000009**Expected MW: 37 kDa*

HeLa cells were treated with 2 μ M 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 μ g) was mixed with [NuPAGE™ LDS Sample Buffer \(4X\)](#), heated at 80 °C for 10 min, and subjected to electrophoresis on [NuPAGE™ 4-12% Bis-Tris, 1.0-1.5 mm Mini Protein Gels](#) in a [Mini Gel Tank](#). Thermo Fisher™ [PageRuler™ Prestained Protein Ladder, 10-180 kDa](#), was used as a molecular weight marker.

Following electrophoresis, 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 1X PBS. The blocked membrane was incubated with Rabbit anti-Human APE1 Polyclonal, IgG for 20 hours, followed by 5 washes with 1X PBS. Identifyn™ SRM Alexa Fluor™ 647 Affinity Purified Donkey 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 $\pm$ 37 nm
Detector = PMT
Intensity = L3
Pixel size = 100 μ m
Scan Speed = Highest
Quality = High
Focus = Membrane (0 μ m in Z-axis)

Post Processing

NONE

Image: S. Khanal

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