Identifyn Standards for Optical Microscopy Methods™
Identifyn® Primary Antibody
Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N)
Cat ID# - PA-RR 000009
Expected MW: 21 kDa

HeLa cells were treated with 2 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. 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 Identifyn® Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) for 20 hours, followed by five 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-IR and Z-Plane Imaging.

Scan Settings

Detection mode = Fluorescence
Laser = 632 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: K. Small

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