Identifyn Standards for Optical Microscopy Methods™
Identifyn® Primary Antibody
Rabbit anti-Human Chk1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Expected MW: 55 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™ 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.

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 Chk1 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 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 Imaging.

Scan Settings

Detection mode = Fluorescence
Laser = 638 nm
Emission Filter = 676 ± 37 nm
Detector = PMT
Intensity = L2
Pixel size = 100 µm
Scan Speed = Highest
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

NONE

Image: S. Khanal

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