

Identifyn Standards for Optical Microscopy Methods™Identifyn Primary Antibody**Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate***Cat ID# - DC 000020*

HEK 293 cells were damaged with 200uM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer and then centrifuged to pellet down the cell debris. The lysate supernatant (100ug) 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 and washed 5X with PBS. The blocked membrane was incubated with Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate for 2 hours, followed by 5X PBS washes. The membrane was dried for 10 minutes at room temperature before imaging.

[Control Data - Primary Unconjugated Antibody](#)

Scanner = [Azure Sapphire FL](#) with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane(s)

Scan Settings

Detection mode = Fluorescence
Laser = 638nm
Emission Filter = 676±37nm
Detector = PMT
Intensity = 2
Pixel size = 100 μ m
Scan Speed = High
Quality = High
Focus = Membrane (0 μ m in Z-axis)

Post Processing

None

Image: Kyle Small

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