

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human γ H2Ax (Serine 139) Recombinant Monoclonal, Mouse Fc, IgG2a***Cat ID - PA-RR 000005**Expected MW: 15 kDa*

HeLa cells were treated with 200 μ M etoposide for 24 hours to induce DNA damage. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer and 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 using [NuPAGE™ 4 to 12% Bis-Tris, 1.0–1.5 mm, Mini Protein Gels](#) in a [Mini Gel Tank](#). The ThermoFisher'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 then washed five times with PBS. The blocked membrane was incubated with Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Mouse Fc, IgG2a, for 2 hours, followed by five washes in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, 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(s)

Scan Settings

Detection mode = Fluorescence

Laser = 488nm

Emission Filter = 513 $\pm$ 17nm

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: K. Small

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