

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody.

Rabbit anti-Human γ H2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)

Cat ID# - PA-RR-000004

Etoposide-treated (2 μ M) HeLa cells were homogenized using an ultrasonication probe and centrifuged to pellet the cell debris. The lysate supernatant 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 ThermFisher'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 Identifyn® Rabbit Anti-Human γ H2AX chimeric antibody (Human Fc) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat anti-Human IgG was incubated for 1 hour, followed by washing 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 = 532nm
Emission Filter = 624 $\pm$ 40nm
Detector = Avalanche Photodiode
Intensity = 6
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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