

Methods

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

Cat ID# - PA-RR-000001

HeLa cells, induced with 2 μ M Etoposide over 24 hours for DNA damage and control non-damaged, were lysed and homogenized with an ultrasonicator probe. The supernatant of these lysates was mixed with [NuPAGE™ LDS Sample Buffer \(4X\)](#) and subjected to [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 for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat anti-Rabbit, F(ab')₂ (H+L) was incubated for 1 hour and washed 5X with PBS. The membrane was dried for 10 min at room temperature before imaging.

Scanner = [Azure Sapphire FL](#) using Confocal scanning with lasers and PMT detectors

Scan Settings

Detection mode = Fluorescence

Laser = 638 / 676BP37

Intensity = 2

Pixel size = 100 μ m

Scan Speed = High

Quality = High

Focus = Membrane (0 μ m in Z-axis)

Post Processing

Light Adjustment

Black = 6957

White = 24239

Gamma = 1.14

Summary

Immunofluorescent Western Blot demonstrates a sparse protein band before etoposide-induced DNA damage, with a robust signal following treatment, confirming Rabbit anti-Human γ H2Ax Recombinant Monoclonal ID# - PA-RR 000001 is highly target specific.

Image: Identifyn® LLC

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