

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human 53BP1 Polyclonal, IgG***Cat ID# - PA-RP 000008**Expected MW: 214 kDa*

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and 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 using [NuPAGE™ Tris-Acetate Mini Protein Gels, 3 to 8%, 1.0 mm](#) in a [Mini Gel Tank](#). The Thermo Fisher's [HiMark™ Pre-stained Protein Standard](#) 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 53BP1 Polyclonal, IgG for 20 hours, followed by 5X PBS washes. Identifyn™ SRM Alexa Fluor™ 647 Affinity Purified Goat 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(s)

Scan Settings

Detection mode = Fluorescence

Laser = 632nm

Emission Filter = 676±37nm

Detector = PMT

Intensity = 3

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: K. Small

© Copyright 2026 GMD12 LLC.