

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human PARP1 Recombinant Monoclonal - (BLR018M)***Cat ID# - PA-RR 000003**Expected Molecular Weight: 116 kDa*

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing.

This antibody was produced against Synthetic Peptides Corresponding to Variable Regions within Human PARP1. While we see a clear DNA damage response, we also note the presence of native PARP1, albeit at a lower concentration in the non-damage lane.

Method -

HEK293 cells were damaged with 200 µM 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 (100 µg) 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 then washed five times with PBS. The blocked membrane was incubated with Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG for 2 hours, followed by five washes in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 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 = 488nm

Emission Filter = 513±17nm

Detector = PMT

Intensity = 2

Pixel size = 100 µm

Scan Speed = High

Quality = High
Focus = Membrane (0 μm in Z-axis)

Post Processing

None

Image: Kyle Small
Data Presentation by P.D. Amistoso

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