

Identifyn Standards for Optical Microscopy Methods™**Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse IgG (H + L)**
Cat ID# - SA 000015

HeLa cells were homogenized using an ultrasonication probe and centrifuged to pellet down 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 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 [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 [Actin Monoclonal Antibody \(mAbGEa\) \(MA1-744\)](#) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey anti-Mouse IgG (H+L) 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 = 488nm
Emission Filter = 513±17nm
Detector = PMT
Intensity = 7
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Identifyn® LLC

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