

Identifyn Standards for Optical Microscopy Methods™**Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse IgG (H + L)***Cat ID# - SA 000015***Identifyn® SRM Alexa Fluor™ 750 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000079*

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with [Thermo Fisher, Fish Serum Blocker cat#37527X3](#). The first primary antibody, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, [Actin Recombinant Rabbit Monoclonal Antibody \(JF47-01\)](#), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 750 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using [Prolong™ Diamond Antifade Mountant cat#36961](#).

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.110µm X 0.110µm

Image size (Pixels) = 1973 X 1705

Image Size (Scaled) = 108.05µm X 93.37µm

Bit Depth = 16 Bit

Image Center Position = X: 0.00µm, Y: 0.00µm

ROI Center Offset = X: 0.00µm, Y: 0.00µm

488 -

Reflector = 38 HE eGFP

Beam Splitter = 495

Filter Excitation Wavelength = 450 - 490

Filter Emission Wavelength = 500 - 550

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @8%

Exposure Time = 100ms

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.4

Imaging Device = AxioCam 705

Camera Adapter = 1X

Depth of Focus = 0.80µm

Binning Mode = 1,1

750 -

Reflector = AF750-49007
Beam Splitter = 760
Filter Excitation Wavelength = 660 - 745
Filter Emission Wavelength = 765 - 855
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @40%
Exposure Time = 800ms
Excitation Wavelength = 752
Emission Wavelength = 779
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.21µm
Binning Mode = 1,1

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 25ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 1,1

Post Processing

None

Image Correction

None

Image by J.K. Bennett

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