

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 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 primary antibody, [Actin Monoclonal Antibody \(mAbGEa\)](#), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 488 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 with DAPI cat#P36962](#).

Microscope

Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D)***Z-Stack = 181 Slices (9.0µm)***

Channels = 2

Scaling (Per Pixel) = 70.560nm X 70.60nm X 50.00nm

Image Size (Pixels) = 1026 X 1026

Image Size (Scaled) = 72.44µm X 72.44µm

Bit Depth = 16 Bit

Image Center Position = X: 78.48mm, Y: 51.50mm

Stage Position = X: 78.48mm, Y: 51.50mm

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

488 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00AU / 45µm

LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)

Laser Wavelength = 488nm @1.0%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 1.4

Rotation = 0°

Sampling = 1.00

Pixel Time = 0.51µs

Frame Time = 5.07s

LSM Scan Speed = 9

Scan Direction = Bidirectional

Line Step = 1

Averaging - 8

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.4
Detection Wavelength = 484 - 590
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

DAPI -

Reflector - None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 37µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.51µs
Frame Time = 5.07s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410-470
Imaging Device = GaAsp-PMT1
Detector Type = GaAsp-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% (488), Ramp 0% (DAPI),
Maximum 100% all channels
Background = Black
Light = Brightness100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph
NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by J.K. Bennett

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