Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human, ATRIP Monoclonal, IgG2b - (4H6H4/G3)
Cat ID# - PA-MM 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000022

ATRIP (ATR Interacting Protein) is a key partner and co-activator of ATR (Ataxia Telangiectasia and Rad3-related), a crucial kinase in the DNA damage response, particularly in response to replication stress and single-strand breaks (SSBs). ATRIP plays a pivotal role in recognizing and binding to single-stranded DNA (ssDNA), facilitating ATR activation at DNA damage sites.

Overview of ATRIP

ATRIP is the regulatory subunit that associates with ATR and directs it to sites of DNA damage. It contains a domain that allows it to bind to Replication Protein A (RPA), a complex that binds and stabilizes single-stranded DNA. This interaction with RPA-coated ssDNA is crucial for ATRIP's ability to guide ATR to regions where the DNA damage response is needed.

Role of ATRIP in Single-Strand Break Repair

ATRIP plays a central role in the activation of ATR, which is critical to the cellular response to single-strand breaks and replication stress:

Recognition of Single-Stranded DNA: ATRIP, through its interaction with RPA, identifies regions of ssDNA. These regions are commonly found at stalled replication forks, resected DNA ends, or sites of single-strand breaks.

Recruitment of ATR: Once ATRIP binds to RPA-coated ssDNA, ATR is recruited to the site. This recruitment is crucial for ATR activation, leading to a cascade of events that coordinate the DNA damage response and facilitate DNA repair.

Activation of Checkpoints: By facilitating ATR activation, ATRIP indirectly contributes to checkpoint signaling. This leads to CHK1 phosphorylation, which plays a role in cell cycle arrest, providing time for the repair of single-strand breaks before the cell cycle progresses.

ATRIP's Broader Role in the DNA Damage Response

Beyond its specific role in single-strand break repair, ATRIP's interaction with ATR has broader implications for the DNA damage response:

© Copyright 2026 GMD12 LLC.

Coordination of DNA Repair: The ATR/ATRIP complex orchestrates the recruitment of DNA repair proteins. This coordination ensures that repair processes like base excision repair (BER) and homologous recombination can proceed efficiently at sites of single-strand breaks.

Response to Replication Stress: ATRIP is crucial in responding to replication stress, which can lead to single-strand breaks or stalled replication forks. By facilitating ATR activation, ATRIP helps stabilize replication forks and prevent more severe DNA damage, such as double-strand breaks.

ATRIP is a critical regulatory protein that guides ATR to sites of single-strand breaks and other forms of replication stress. By recognizing RPA-coated ssDNA and recruiting ATR, ATRIP plays a pivotal role in activating the DNA damage response, leading to checkpoint activation and coordination of DNA repair processes. Its role in stabilizing replication forks and promoting genomic stability underscores its importance in maintaining genome integrity.

Method

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, Mouse anti-Human, ATRIP Monoclonal, IgG2b - (4H6H4/G3), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

The second primary antibody, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature 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 980 Confocal **with NIR Detection**, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Single Plane (2D) - Z-Plane 74 of 141 - *Panels A & E*

Z Stack - (3D) - *Panel B*

Z-Stack = 141 Slices (4.2 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1121 X 1121
Image Size (Scaled) = 65.93 µm X 65.93 µm
Bit Depth = 16 Bit
Image Center Position = X: 83.24 mm, Y: 49.90 mm
Stage Position = X: 83.24 mm, Y: 49.90 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

© Copyright 2026 GMD12 LLC.

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 65µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = **639nm @0.7%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.30µs
Frame Time = 3.69s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 668
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.8 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **488nm @0.5%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.30µs
Frame Time = 3.69s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 493
Emission Wavelength = 517

© Copyright 2026 GMD12 LLC.

Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 43µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = **405nm @0.2%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.30µs
Frame Time = 3.69s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430 - 470
Imaging Device = GaAsp-Pmt3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.5 (3D, Auto)

2=Dimensional View - *Panel A*

Panel features Mouse anti-Human, ATRIP Monoclonal, IgG2b - (4H6H4/G3) with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific (647 psuedo colored violet, instead of default red in Zeiss Zen), combined with a Rabbit Recombinant Actin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), (psuedo colored white, instead of the default green in Zeiss Zen).

Image was taken from Z-stack Acquisition at plane 71 of 141

3D Image Processing - *Panel B*

© Copyright 2026 GMD12 LLC.

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, 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)

Note that the Actin, 488 staining was pseudo-colored red from green in Zeiss Zen, and the ATRIP, 647 staining was from the default red to white. DAPI is dark blue.

LSM 980 Zen Acquisition Info Tab - *Panel C*

Shown is the acquisition of the 647 channel (ATRIP Mouse Monoclonal with Identifyn 647 secondary) using the **639nm laser at 0.7%,** the 488 channel (ACTIN) using the **488nm laser at 0.5%**, and DAPI using the **405nm laser at 0.2%**

Profile View Display - *Panels D & E*

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 42.3), Y (Min 0 and Max 14933)

This panel measures ATRIP DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately 15K A.U. at its maximum.

Measured at Z-plane 71 of 141

Histogram Display - *Panels E & F*

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 50000)

The histogram was generated from the yellow-boxed region shown in Panel E to quantify the overall ATRIP protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed signal increase relative to the control condition (see control), complementing the line-profile analysis presented alongside it.

Image Corrections

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from yellow, the default color, to dark grey to provide visual contrast in Panel A.

Image by J.K. Bennett

© Copyright 2026 GMD12 LLC.

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

IDENTiFYN®