

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

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:

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. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, 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 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI cat#P36962](#).

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 28 of 55 total - Panel A

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1443 X x 1784
Image Size (Scaled) = 30.47 µm X 37.66 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.85 mm, Y: 39.09 mm
Stage Position = X: 57.85 mm, Y: 39.09 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) and Z-Stack (3D) - Z-plane 28 of 55 total - Panels B and C, ROI

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 371 X x 352
Image Size (Scaled) = 7.83 µm X 7.43 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.85 mm, Y: 39.09 mm
Stage Position = X: 57.85 mm, Y: 39.09 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel D

Z-Stack = 55 Slices (1.62 µm)

Channels = 2

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 126 X x 126

Image Size (Scaled) = 2.66 µm X 2.66 µm

Bit Depth = 16 Bit

Image Center Position = X: 57.85 mm, Y: 39.09 mm

Stage Position = X: 57.85 mm, Y: 39.09 mm

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

647 -

Reflectors = SR HE LBF 405/488/561/642

Beam Splitter = 405, 488, 561, 642

Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642

Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Contrast Method = Fluorescence

Illumination Wavelength = 640

Channel Name = T1-Axiocam 820-SR

Excitation Wavelength = 640

Emission Wavelength = 729

Effective NA = 1.4

Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Imaging Device = Axiocam 820

Camera Adapter = 1X

Exposure Time = 120 ms

Depth of Focus = 1.13 µm

Binning Mode = 2,2

Laser Wavelength = 640 nm @1.00%

Frame Time = 1.59 s

Scan Direction = Unidirectional

Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642

Beam Splitter = 405, 488, 561, 642

Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642

Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Contrast Method = Fluorescence

Illumination Wavelength = 405

Channel Name = T3-Axiocam 820 #2-SR

Excitation Wavelength = 405

Emission Wavelength = 445

Effective NA = 1.4

Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Imaging Device = Axiocam 820 #2

Camera Adapter = 1X

Exposure Time = 50 ms

Depth of Focus = 0.69 µm

Binning Mode = 2,2
Laser Wavelength = 405 nm @2.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 μ m grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 11.1252 (647), 10.0685 (DAPI)
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel D

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 120%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Three-dimensional volume projection showing ATRIP detected with an Identifyn® mouse monoclonal antibody and visualized using an Identifyn® SRM Alexa Fluor™ 647 goat anti-mouse secondary antibody (red), with DAPI nuclear staining (grey/white). Relative to Airyscan imaging, this modality provides increased spatial resolution and begins to resolve separation within individual ATRIP foci or aggregates, consistent with structures further resolved by STORM imaging.

Image Corrections

DAPI was shown in grey in panels A, B and D, and white in panel B, rather than the default blue, to enhance visual contrast.

Image by P. Raut

Image Analysis and Data Presentation by B.T. Bennett

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