

## 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™ 532 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)**

*Cat ID# - SA 000027*

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, then fixed in -20°C methanol 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 incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 532 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 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 = 3  
Scaling (Per Pixel) = 0.110 µm X 0.110 µm  
Image size (Pixels) = 1216 X 1028  
Image Size (Scaled) = 133.18 µm X 112.59 µm  
Bit Depth = 14 Bit  
Image Center Position = X: 0.00 µm, Y: 0.00 µm  
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

### 647 -

Reflector = 50 Cy 5  
Beam Splitter = 660  
Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715  
 Contrast Method = Fluorescence  
 Light source = X-Cite Xylis Lamp Intensity @15.00%  
 Exposure Time = 150 ms  
 Excitation Wavelength = 653  
 Emission Wavelength = 668  
 Effective NA = 1.4  
 Imaging Device = Axiocam 705  
 Camera Adapter = 1X  
 Depth of Focus = 1.03  $\mu\text{m}$   
 Binning Mode = 2,2

### 532-

Reflector = AF532-49014  
 Beam Splitter = 550  
 Filter Excitation Wavelength = 515 - 545  
 Filter Emission Wavelength = 555 - 595  
 Contrast Method = Fluorescence  
 Light source = X-Cite Xylis Lamp Intensity @10.00%  
 Exposure Time = 100 ms  
 Excitation Wavelength = 528  
 Emission Wavelength = 553  
 Effective NA = 1.4  
 Imaging Device = Axiocam 705  
 Camera Adapter = 1X  
 Depth of Focus = 0.86  $\mu\text{m}$   
 Binning Mode = 2,2

### 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 @10.00%  
 Exposure Time = 25 ms  
 Excitation Wavelength = 353  
 Emission Wavelength = 465  
 Effective NA = 1.4  
 Imaging Device = Axiocam 705  
 Camera Adapter = 1X  
 Depth of Focus = 0.72  $\mu\text{m}$   
 Binning Mode = 2,2

### Split View - Panel A

The top-left panel shows mouse anti-human ATRIP monoclonal antibody (IgG2b; clone 4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 647 affinity-purified goat anti-mouse IgG (H+L; subclasses 1, 2a, 2b, and 3), Fcy fragment-specific secondary antibody. The top-right panel shows rabbit recombinant actin, visualized using Identifyn® SRM

Alexa Fluor™ 532 affinity-purified donkey anti-rabbit IgG (H+L). The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

### **Colocalization View - Panel B & C**

Horizontal Axis - AF647, Threshold 97, Range Auto

Vertical Axis - DAPI, Threshold 2132, Range Auto

Image Measurement = Entire Field

Colocalization analysis of ATRIP (X-axis) and DAPI (Y-axis) was performed across the entire image acquisition. ATRIP shows strong colocalization with DAPI, consistent with localization within the nuclear compartment.

### **Image Correction**

Identifyfyn® SRM Alexa Fluor™ 532 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.

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Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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