

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human Chk1 Polyclonal, IgG***Cat ID# - PA-RP 000011***Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3)***Cat ID# - PA-MM 000003*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000049***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific***Cat ID# - SA 000012*

Chk1 (Checkpoint Kinase 1) is a critical serine/threonine kinase involved in the cellular response to DNA damage, particularly in checkpoint activation and cell cycle regulation. It significantly prevents cells from progressing through the cell cycle when DNA damage remains unrepaired, including single-strand breaks (SSBs). Here's an overview of Chk1's role in single-strand break repair and in maintaining genomic stability.

Overview of Chk1

Chk1 is a key downstream effector of the ATR (Ataxia Telangiectasia and Rad3-related) pathway, which is activated in response to replication stress and single-strand breaks. Upon activation by ATR, Chk1 coordinates the cellular response by signaling for cell cycle arrest, stabilizing replication forks, and promoting DNA repair.

Role of Chk1 in Response to Single-Strand Breaks

Chk1's role in single-strand break repair involves several vital functions, which contribute to the broader DNA damage response:

Cell Cycle Checkpoint Activation: When ATR is activated in response to single-strand breaks or replication stress, it phosphorylates Chk1. This phosphorylation triggers checkpoint activation, leading to cell cycle arrest or delay. This pause in the cell cycle allows DNA repair processes to fix the damage before the cell replicates or divides.

Regulation of Replication Fork Progression: Chk1 plays a crucial role in stabilizing and regulating replication forks, preventing them from collapsing into double-strand breaks. This is especially important when single-strand breaks or other forms of damage occur during DNA replication.

Coordination of DNA Repair: Chk1 provides a window for DNA repair pathways to operate by activating cell cycle checkpoints. This coordination ensures that repair proteins involved in base excision repair (BER) and other pathways have sufficient time to correct single-strand breaks and prevent further genomic instability.

Chk1's Role in Broader DNA Damage Response

Beyond its direct role in responding to single-strand breaks, Chk1 contributes to the overall DNA damage response:

Cross-Talk with Other Pathways: Chk1's activation can lead to interactions with other signaling pathways, including p53 and other cell cycle regulators. This cross-talk helps coordinate a broader response to DNA damage, ensuring genomic stability.

Promotion of Genomic Stability: Chk1 helps maintain genomic stability by preventing cells with unrepaired DNA from progressing through the cell cycle. This function is crucial in reducing the risk of mutations and chromosomal aberrations.

Chk1 plays a critical role in the cellular response to single-strand breaks by activating cell cycle checkpoints, stabilizing replication forks, and coordinating DNA repair processes. Its role as a downstream effector of the ATR pathway makes it essential for preventing cells with damaged DNA from progressing, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 μ M etoposide-spiked complete growth media for 24 hours, then cells were fixed in -20 °C methanol and blocked with [Thermo Fisher Fish Serum Blocker \(cat#37527X3\)](#). The primary antibody, Rabbit anti-Human Chk1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b - (4H6H4/G3), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X 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 μ m X 0.110 μ m

Image size (Pixels) = 1029 X 889

Image Size (Scaled) = 112.70 μ m X 97.37 μ m

Bit Depth = 14 Bit

Image Center Position = X: 0.00 μ m, Y: 0.00 μ m

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

594 -

Reflector = 45 Texas Red

Beam Splitter = 585

Filter Excitation Wavelength = 540 - 580

Filter Emission Wavelength = 593 - 668

Contrast Method = Fluorescence

Light Source = X-Cite Xylis Lamp Intensity @ 15.00%

Exposure Time = 150 ms
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1x
 Depth of Focus = 0.96 μm
 Binning Mode = 2,2

488 -

Reflector = 38 eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light Source = X-Cite Xylis Lamp Intensity @ 20.00%
 Exposure Time = 200 ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1x
 Depth of Focus = 0.80 μm
 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 = 20 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1x
 Depth of Focus = 0.72 μm
 Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Rabbit anti-Human Chk1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 affinity-purified Donkey anti-Rabbit IgG (H+L). The top-right panel shows Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 488 affinity-purified Goat anti-Mouse IgG (H+L). The lower-left panel shows DAPI nuclear staining, and the bottom-right panel shows the three-channel merged image.

2.5D Image Processing - Panel B

2.5D Display

Render Mode = Surface

Grid distance = 5

Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 54

Angle Y = -180

Z Scaling = 1.2

Ambient = 50

Specular = 20

Shininess = 50

Light Height = 2.0

Colocalization View - Panel C

Horizontal Axis - AF594, Threshold 250, Range Auto - Chk1

Vertical Axis - AF488, Threshold 396, Range Auto - ATRIP

Image Measurement = Entire Field

Colocalization analysis of Chk1 (X-axis) and ATRIP (Y-axis) was performed across the entire image acquisition.

Summary

Human Chk1 Polyclonal, IgG, visualized using Identifyn[®] Donkey anti-Rabbit 594 Secondary. In contrast, the upper right panel shows Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3) visualized using Identifyn[®] Goat anti-Mouse 488 Secondary. The lower-left panel shows DAPI nuclear staining, and the lower-right panel shows the merged three-channel image. Together, these views demonstrate overlapping intranuclear organization and apparent colocalization between Chk1 and ATRIP following DNA damage induction.

Panel B presents a 2.5D reconstruction generated in Zeiss ZEN software from the three cells contained within the acquisition field. While widefield microscopy lacks the resolving power to definitively distinguish molecular-level colocalization, the reconstruction reveals interspersed nuclear distributions between the Chk1/594 and ATRIP/488 signals, suggesting coordinated recruitment within shared DNA damage-associated regions.

Panel C shows colocalization analysis of the same acquisition, with ATRIP intensity plotted on the vertical axis and Chk1 intensity on the horizontal axis. Strongly correlated localization is observed between the two markers, supporting coordinated spatial organization in response to etoposide-induced replication stress and DNA damage signaling.

Biologically, ATRIP functions as the obligate binding partner of ATR and is recruited to regions of RPA-coated single-stranded DNA generated during replication stress and DNA damage processing. Upon recruitment, the ATR-ATRIP complex initiates checkpoint signaling by phosphorylating and activating Chk1, a central effector kinase that coordinates cell-cycle arrest, replication fork stabilization, and DNA repair progression.

The observed intranuclear overlap between ATRIP and Chk1 is therefore consistent with activation of the ATR-mediated replication stress response pathway. It supports their coordinated engagement within DNA damage signaling microenvironments.

Image Correction

NONE

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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