

Identifyfyn Standards for Optical Microscopy Methods™Identifyfyn® 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*Identifyfyn® Secondary Antibodies**Identifyfyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000049***Identifyfyn® 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 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:

Z Stack - (3D) - Panel A, Z-Plane 81 of 161 **Z-Stack = 161 Slices (4.8 μ m)**

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 766 X 683
Image Size (Scaled) = 27.04 μ m X 24.11 μ m
Bit Depth = 16 Bit
Image Center Position = X: 75.22 mm, Y: 49.76 mm
Stage Position = X: 75.22 mm, Y: 49.76 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Panel B, Volume Projection **Z-Stack = 161 Slices (4.8 μ m)**

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm

Image Size (Pixels) = 164 X 147
Image Size (Scaled) = 5.79 μm X 5.19 μm
Bit Depth = 16 Bit
Image Center Position = X: 75.22 mm, Y: 49.76 mm
Stage Position = X: 75.22 mm, Y: 49.76 mm
ROI Center Offset = X: 0.00 μm , Y: 0.00 μm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 286 μm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS LP 525
Laser Wavelength = **561 nm @ 3.50%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15 μs
Frame Time = 17.86 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 573 - 627
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = Filter: 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 μm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = **488 nm @ 2.1%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15 μs
Frame Time = 17.86 s

LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 4
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 499 - 548
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = Super Resolution: 9.1 (3D, Auto)

2 Dimensional View - Panel A

Shows an LSM980 confocal optical section acquired at Z-plane 81 of 161, demonstrating Rabbit anti-Human Chk1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody, Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3), visualized using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody. A white box shows the region of interest to be examined in panel B.

3D Image Processing

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)

Summary

Panel A shows a single Airyscan optical section at Z-plane 81 of 161 depicting Chk1 and ATRIP distribution following etoposide-induced DNA damage. Rabbit anti-Human Chk1 Polyclonal, IgG is detected using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody (red), and Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3), is detected using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody (green), with DAPI defining the nuclear compartment.

Panel B presents a three-dimensional volume projection of both proteins. Two yellow-circled regions highlight aggregate formations that visually correspond to similar structures observed in the STORM image shown in Panel C. At Airyscan resolution (~120 nm in XY), these structures remain highly conflated and may represent multiple unresolved molecular signals rather than singular protein assemblies. However, the repeated appearance of doublet-like formations suggests possible spatial separation within DNA damage-associated domains, potentially consistent with paired or adjacent repair structures at processed DNA lesions or break-associated signaling regions.

Airyscan can resolve structures laterally to ~120 nm, but remains limited compared with nanoscale localization methods such as STORM.

Panel C shows the corresponding STORM reference image, which resolves finer sub-diffractional organization not fully separable by Airyscan. Together, these data support Airyscan as a valuable Stepdown modality for identifying damage-induced spatial organization of Chk1/ATRIP and candidate aggregate architecture, while confirming that definitive nanoscale structural interpretation requires STORM-level ground-truth imaging.

Biologically, ATRIP recruits and stabilizes ATR signaling at RPA-coated single-stranded DNA generated during replication stress or resected DNA double-strand breaks. At the same time, ATR-dependent signaling activates Chk1 to coordinate replication fork stabilization, checkpoint arrest, and repair progression. The observed Chk1/ATRIP aggregates are therefore consistent with coordinated engagement of the ATR-ATRIP-Chk1 replication stress response following etoposide treatment. While doublet-like patterns may reflect separate break-associated signaling domains, this interpretation should be treated with caution at Airyscan resolution, as DNA repair foci can contain clustered nanoscale substructures that require higher-resolution imaging to be confidently resolved.

Image Correction

NONE

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

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