

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) - Panels A, B, and C, and Showing Z-Plane 74 of 156
Z-Stack = 156 Slices (4.65 μ m)

Channels = 3
 Scaling (Per Pixel) = 0.059 μ m X 0.059 μ m X 0.030 μ m
 Image size (Pixels) = 1612 X 1612
 Image Size (Scaled) = 94.79 μ m X 94.79 μ m
 Bit Depth = 16 Bit
 Image Center Position = X: 81.40 mm, Y: 51.78 mm
 Stage Position = X: 81.40 mm, Y: 51.78 mm
 ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

594 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 59 μ m

LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = **561 nm @ 0.6%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.24 µs
Frame Time = 6.04 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590 - 645
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.9 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 51 µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = **488 nm @ 1.0%**
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.24 µs
Frame Time = 6.04 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 549
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0

Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 7.7 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 44 μ m
 LMS MBS = MBS 488/561 & 405/730
 LMS SBS = SBS LP 525
 Laser Wavelength = **405 nm @ 0.4%**
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.4
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.24 μ s
 Frame Time = 6.04 s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 435 - 490
 Imaging Device = GaAsp-Pmt3
 Detector Type = GaAsp-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.9 (3D, Auto)

2 Dimensional View - Panel A

Shows an LSM980 confocal optical section acquired at Z-plane 74 of 156 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, and DAPI nuclear staining. A line profile traversing the lower nucleus measures fluorescent intensity across all three channels.

Profile View Display - Panels B and C

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 65.0), Y (Min 0 and Max 27903)

Measured at Z-plane 74 of 156

Zen Acquisition Info Tab - Panel C

Panel C shows the Zeiss ZEN acquisition parameters used for image collection, including 0.6% laser power for the 561 nm channel (Alexa Fluor™ 594), 1.00% laser power for the 488 nm channel (Alexa Fluor™ 488), and 0.4% laser power for the 405 nm DAPI channel.

Summary

Panel A shows an LSM980 confocal optical section acquired at Z-plane 74 of 156, 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, and DAPI nuclear staining. A line profile traversing the lower nucleus measures fluorescent intensity across all three channels, demonstrating that Chk1, ATRIP, and DAPI signals remain confined to the nuclear compartment. The line profile additionally reveals punctate regions of elevated Chk1 intensity associated with intranuclear aggregate formation following etoposide-induced DNA damage.

Panel B shows histogram-based quantitative analysis of the measured fluorescent intensities from the line profile shown in Panel A. Four prominent Chk1-associated aggregate regions were identified with measured fluorescent intensities of 26,674.00, 20,159.00, 18,045.00, and 15,956.00 arbitrary units (A.U.), respectively. These findings support localized redistribution and concentration of Chk1 into discrete intranuclear signaling domains rather than a uniform increase in total nuclear protein abundance. While Chk1 is constitutively distributed throughout the nucleus under basal conditions, DNA damage and replication stress promote its spatial reorganization into punctate repair-associated microenvironments, coordinated by ATR-ATRIP signaling.

Panel C provides acquisition transparency by including the Zeiss ZEN imaging parameters used to collect the dataset. Recorded excitation inputs included 0.6% laser power for the 561 nm channel corresponding to Alexa Fluor™ 594, 1.00% laser power for the 488 nm channel corresponding to Alexa Fluor™ 488, and 0.4% laser power for the 405 nm DAPI channel.

Biologically, ATRIP functions as the obligate recruitment partner of ATR and localizes to regions of RPA-coated single-stranded DNA generated during replication stress and DNA end resection. Upon recruitment, ATR-mediated phosphorylation activates Chk1, initiating checkpoint signaling pathways that stabilize the replication fork, trigger intra-S phase arrest, and coordinate DNA repair. The observed intranuclear aggregation and colocalization patterns are therefore consistent with activation of the ATR-ATRIP-Chk1 replication stress response pathway following etoposide exposure. Importantly, the data suggest that the observed Chk1 response reflects redistribution and focal enrichment within damage-associated signaling domains rather than wholesale increases in total nuclear Chk1 protein volume, a finding consistent with the established biology of checkpoint kinase activation.

Image Correction

NONE

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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