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:

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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 µM 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 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:

Z Stack - (3D) - *Panel A, Z-Plane 67 of 134*
Z-Stack = 134 Slices (3.99 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1694 X 1267
Image Size (Scaled) = 35.76 µm X 26.75 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.98 mm, Y: 35.05 mm
Stage Position = X: 64.98 mm, Y: 35.05 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - *Panel B, Volume Projection*

Z-Stack = 134 Slices (3.99 µm)

Channels = 2

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Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 239 X 219
Image Size (Scaled) = 5.05 µm X 4.62 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.98 mm, Y: 35.05 mm
Stage Position = X: 64.98 mm, Y: 35.05 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

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 = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 200 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @ 15.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 32.5 µm grating

488 -

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 = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 503 - 546, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @ 5.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional

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Grating = 27.5 µm 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 = 11.1066 (594), 11.0384 (488)
Fitting = Fast Fit
Normalization = Auto

2 Dimensional View - *Panel A*

Shows an Elyra 5, Lattice SIM, optical section acquired at Z-plane 67 of 134, 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 - P*anel B, Showing Chk1/594 only*

3D Volume Projection = Precise
Appearance = Threshold 4%, Ramp 96%, Maximum 100%
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 optical section (Z-plane 67 of 134) 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).

Panel B presents a three-dimensional volume reconstruction of Chk1 from the same region. Regions of interest (ROIs), indicated by yellow circles, highlight concentrated aggregate-like signal patterns that demonstrate superficial similarity to higher-resolution STORM-resolved structures. The observed Chk1 aggregates reflect localized enrichment within DNA damage-associated nuclear compartments; however, the structures remain diffraction-limited and do not resolve discrete molecular subunits. Recurrent doublet-like signal configurations are observed throughout several aggregate regions. They may reflect partially separated DNA damage-associated signaling domains or adjacent repair-associated structures, although these features remain unresolved at this resolution scale.

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Despite improved resolution relative to conventional confocal microscopy, Lattice SIM still limits lateral resolution to ~80 nm and can still conflate closely spaced molecular assemblies into a single apparent structure. As a result, Chk1 signals appear as compact foci or aggregates without fully resolving the underlying nanoscale organization. In contrast, Panel C (STORM) resolves these apparent aggregates into more refined structural architectures, demonstrating that the structures observed in Panels A and B represent broader spatial organization rather than definitive molecular detail.

Biologically, Chk1 functions as a central ATR-dependent checkpoint kinase coordinating replication fork stabilization, intra-S phase arrest, and DNA repair progression following replication stress and DNA damage. Unlike proteins that undergo dramatic recruitment increases following damage induction, Chk1 is constitutively present throughout the nucleus under basal conditions. The observed response is therefore more consistent with the redistribution and focal enrichment of pre-existing nuclear Chk1 pools into replication-stress-associated signaling microenvironments rather than with substantial increases in total protein abundance. ATRIP, shown in Panel A, functions as the obligate recruitment partner of ATR and localizes to RPA-coated single-stranded DNA, where it facilitates ATR-mediated activation of Chk1 during the DNA damage response.

Image Correction

NONE

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

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