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:

© Copyright 2026 GMD12 LLC.

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 = 148 Slices (4.41 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1374 X 1522
Image Size (Scaled) = 29.01 µm X 32.13 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.52 mm, Y: 37.48 mm
Stage Position = X: 64.52 mm, Y: 37.48 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) - *Gallery was Reduced to Highlight Signal*

Channels = 2

© Copyright 2026 GMD12 LLC.

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 285 X 288
Image Size (Scaled) = 6.02 µm X 6.08 µm
Bit Depth = 16 Bit
Image Center Position = X: 64.52 mm, Y: 37.48 mm
Stage Position = X: 64.52 mm, Y: 37.48 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

© Copyright 2026 GMD12 LLC.

Grating = 27.5 µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 2
Algorithm = SIM2
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
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 - *Panel B, Showing Chk1/594 only*

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
Background = Black
Light = Brightness 65%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 45°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A shows the same Lattice SIM² optical section described above, with Rabbit anti-Human Chk1 Polyclonal, IgG detected using Identifyn® SRM Alexa Fluor™ 594 Donkey anti-Rabbit secondary antibody (red), and Mouse anti-Human ATRIP Monoclonal, IgG2b – (4H6H4/G3), detected using Identifyn® SRM Alexa Fluor™ 488 Goat anti-Mouse secondary antibody (green), following etoposide-induced DNA damage.

Panel B presents Chk1/594 alone in its default red channel at Lattice SIM² resolution, approximately ~40 nm in XY. At this resolution, Chk1 aggregate substructure becomes more visually apparent, with abundant doublet-like formations observed throughout the nuclear damage response pattern. These doublets may represent spatially separated signaling domains at or near opposing DNA break ends; however, this interpretation should be approached with caution. Even at ~40 nm XY resolution, the Chk1 signal may still represent conflated protein assemblies, antibody-labeled complexes, or clustered checkpoint domains rather than discrete molecular break-end pairs.

© Copyright 2026 GMD12 LLC.

This limitation is especially important given the physical scale of Chk1 itself. Chk1 is approximately ~55 kDa, which corresponds to an estimated globular protein diameter of roughly ~5 nm. Even before accounting for antibody linkage distance and fluorophore position, a single Chk1 molecule is therefore far below the resolving capacity of Lattice SIM². A ~40 nm XY feature could theoretically contain multiple Chk1 molecules and associated signaling partners within a single unresolved structure. Thus, what is being visualized is not individual Chk1 protein, but damage-induced Chk1-enriched nuclear microdomains.

Panel C shows STORM imaging, in which sub-diffractional organization is more clearly resolved. Across the Stepdown Methodology™, the progression from Airyscan to Lattice SIM to Lattice SIM² demonstrates increasing ability to detect Chk1 redistribution, aggregate formation, and candidate colocalization patterns. However, even Lattice SIM² remains limited for defining molecular structure or intimate protein–protein relationships. The strongest conclusion is that Chk1 undergoes damage-induced spatial reorganization into checkpoint-associated nuclear domains, while STORM remains required to resolve true nanoscale subunit architecture.

Even STORM, at an approximate ~20 nm resolution, may still struggle to resolve individual Chk1 molecules or define true molecular boundaries, given that Chk1 is ~55 kDa and estimated to occupy only ~5 nm as a globular protein. Therefore, even STORM-resolved substructures likely represent nanoscale Chk1-enriched signaling domains, clustered assemblies, or antibody-accessible repair microenvironments rather than single-protein events.

Accordingly, the Stepdown Methodology™ is strongest for measuring response, redistribution, aggregate formation, and relative spatial organization across imaging modalities. It can support colocalization and structural inference, but it should not be overinterpreted as direct visualization of individual Chk1 molecules, opposing break ends, or definitive protein–protein interaction without orthogonal validation.

Image Correction

NONE

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

© Copyright 2026 GMD12 LLC.