Identifyn Standards for Optical Microscopy Methods™
Identifyn® Primary Antibody
Rabbit anti-Human CHK1 Polyclonal, IgG
Cat ID# - PA-RP 000011

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000049

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.

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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
Plus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 µM Etoposide–spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Chk1 Polyclonal, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in 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 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Minus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human Chk1 Polyclonal, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in 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 5X in 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) - *Showing Z-Plane 79 of 156, Plus Etoposide*
Z-Stack = 156 Slices (4.65 µm)

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 512 X 572
Image Size (Scaled) = 30.11 µm X 33.64 µm
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 µm, Y: 0.00 µm

Single Plane (2D) - *Showing Z-Plane 75 of 161, Plus Etoposide*

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 492 X 519
Image Size (Scaled) = 28.93 µm X 30.52 µm
Bit Depth = 16 Bit

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Image Center Position = X: 91.81 mm, Y: 52.99 mm
Stage Position = X: 91.81 mm, Y: 52.99 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594- *Plus Etoposide-Induced DNA Damage, from Confocal Data Set*

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 59 µm
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)

594- M*inus Damage - noting the same laser input power and conditions*

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 59 µm
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

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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: 8.3 (3D, Auto)

Profile View Display - *Plus DNA Damage, Top*

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 31.0), Y (Min 0.0 and Max 25515)**

This panel measures Chk1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~57K A.U. at its maximum.

Measured at Z-plane 79 of 156

Profile View Display - *Minus DNA Damage, Bottom*

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - **X (Min 0.0 and Max 28.9), Y (Min 0.0 and Max 19430)**

This panel measures Chk1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~45K A.U. at its maximum.

Histogram Display - Plus DNA Damage, Top

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 1000000)

Arithmetic Mean intensity = 2,679.910

The histogram was generated from the yellow-boxed region shown to quantify the overall Chk1 protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - *Minus DNA Damage, Bottom*

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Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 2000000)

Arithmetic Mean intensity = 1,516.305

The histogram was generated from the yellow-boxed region shown to quantify the overall Chk1 protein within a defined nuclear area in the absence of DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusion –

In the absence of etoposide-induced DNA damage, Chk1 exhibits a predominantly diffuse nuclear distribution, consistent with its constitutive role as a surveillance kinase that regulates replication fidelity and normal cell-cycle progression. Under control conditions, Chk1 signal remains broadly distributed throughout the nucleoplasm with comparatively reduced punctate organization and lower aggregate fluorescent intensity relative to the damage-induced state.

Quantitative line-profile analysis demonstrated that maximal aggregate fluorescent intensity under control conditions reached approximately ~45K A.U., compared with ~57K A.U. following etoposide exposure, representing an approximate ~20% increase in maximal aggregate signal associated with DNA damage induction. Histogram analysis further demonstrated an arithmetic mean intensity of 1,516.305 A.U. in the absence of damage compared with 2,679.910 A.U. following etoposide treatment, supporting an approximate ~45% increase in overall nuclear signal intensity associated with checkpoint activation and spatial reorganization.

Importantly, the observed increase is biologically consistent with redistribution and focal enrichment of pre-existing nuclear Chk1 pools rather than wholesale upregulation of total protein abundance. CHK1 is constitutively expressed and maintained within the nucleus under basal conditions, where it monitors replication integrity and coordinates normal S-phase progression. Following replication stress and activation of the ATR-ATRIP pathway, Chk1 undergoes phosphorylation-dependent relocalization to chromatin-associated signaling domains and stalled replication forks, resulting in punctate aggregate formation and enhanced local signal intensity.

Thus, the control condition establishes the expected baseline diffuse nuclear architecture of Chk1 before checkpoint activation. In contrast, the etoposide-induced condition demonstrates biologically consistent redistribution into damage-associated repair and signaling microenvironments characteristic of the ATR-Chk1 replication stress response pathway.

Image Correction
The Chk1 channel (594) was pseudo-colored white to enhance visual contrast in displaying measurements.

Image by J.K. Bennett

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Image Analysis and Data Presentation by B.T. Bennett

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