

Nu.Q® H3.1 immunoassay: A single assay for monitoring diverse cell death pathways.

Introduction

Cell death is a central feature of tissue homeostasis and a hallmark of numerous physiological and pathological processes, including cancer, inflammation, autoimmune disorders, and treatment response. During cell death mechanisms such as apoptosis, necrosis, and neutrophil extracellular trap formation (NETosis), chromatin is dismantled, leading to the release of nucleosomes into the extracellular environment. As nucleosome release directly reflects chromatin disruption, circulating nucleosomes have emerged as robust biomarkers for monitoring cellular damage and cell death.

The Nu.Q® H3.1 immunoassay quantitatively measures nucleosomes containing histone H3.1, providing a sensitive and convenient approach for assessing cell death in biological samples. Given the assay recognizes H3.1-containing nucleosomes independently of histone H3 post-translational modifications—including methylation, acetylation, phosphorylation, citrullination, and N-terminal tail cleavage—it captures nucleosomes released from multiple cell death pathways, making it a broad and versatile biomarker.

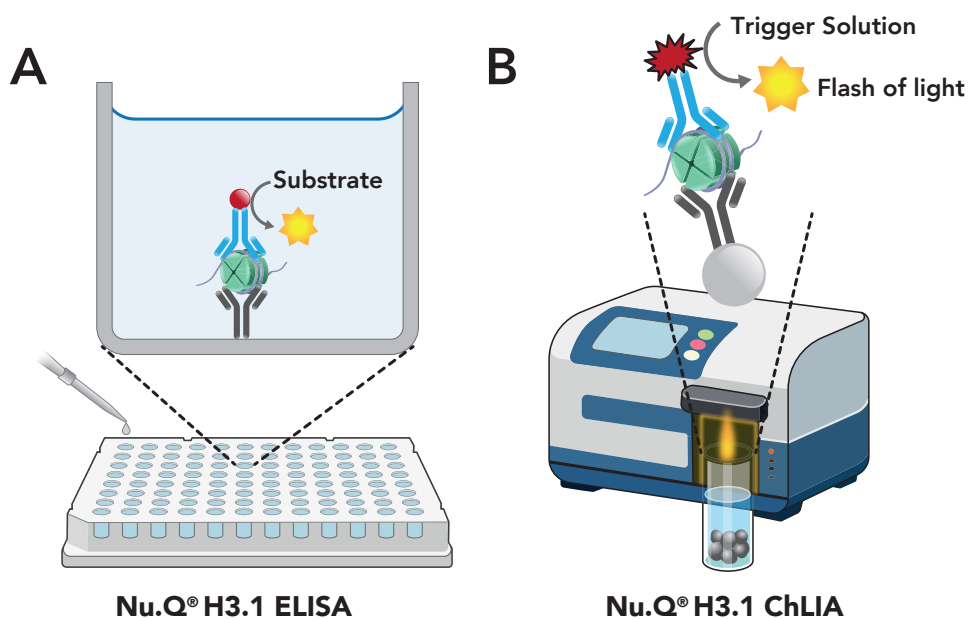
The assay is therefore well suited for a wide range of research and translational applications, including the investigation of disease mechanisms, evaluation of therapeutic responses, and monitoring of tissue damage across experimental and clinical settings.

Results

High-performance immunoassays

Figure 1: Schematic representation of the Nu.Q® H3.1 immunoassay.*

The Nu.Q® H3.1 assay is available on two platforms: A) Manual ELISA, B) Automated chemiluminescence-based Immunoassay (ChLIA) with magnetic particles.



*on automated IDS (Immunodiagnostic Systems Ltd, UK) platforms

The Nu.Q® H3.1 ELISA is based on a sandwich immunoassay using an anti-histone H3.1 capture antibody coated on a microtiter plate, combined with a conformational anti-nucleosome detection antibody conjugated to HRP (Fig. 1A). The assay demonstrated robust analytical performance, with a limit of blank (LOB) of 3.4 ng/mL, a limit of detection (LOD) of 9.1 ng/mL, and a lower limit of quantification (LOQ) of 22.7 ng/mL (Fig. 2). Linearity was confirmed over a range of 22.7 to 500.3 ng/mL using K2EDTA plasma samples (Fig. 3A). Precision analysis showed intra-assay variability ranging from 4.2% to 11.8% and total precision between 8.5% and 19.3% (Fig. 4A). Overall, the ELISA format provides reliable quantification and offers flexibility and accessibility, making it well-suited for research laboratory settings.

	ng/ml		
	LOB	LOD	LOQ
ELISA	3.4	9.1	22.7
ChLIA	0.3	0.9	2.9

Figure 2: Analytical sensitivity of Nu.Q® H3.1 on both platforms ELISA and ChLIA

H3.1-nucleosomes assay limits of blank (LOB), limits of detection (LOD) and limits of quantification (LOQ) from one representative lot of ELISA or ChLIA assay.

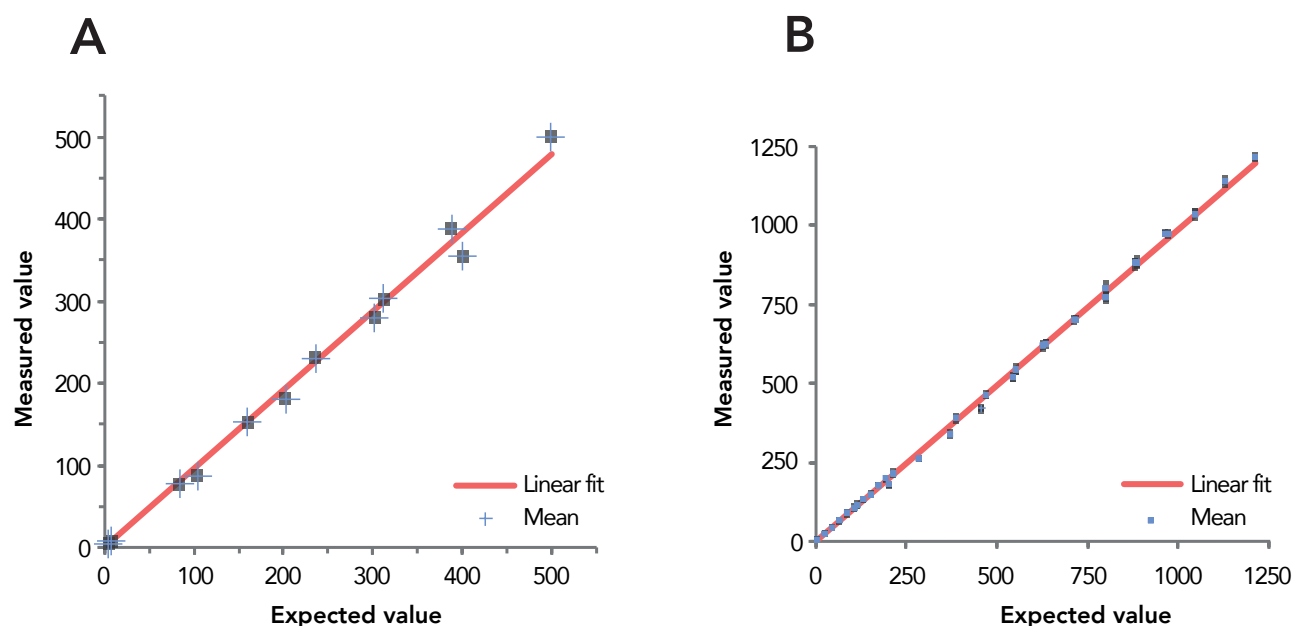


Figure 3: H3.1-nucleosome immunoassays linearity

K2EDTA plasma samples containing low concentrations of H3.1-nucleosomes were mixed with plasma samples containing higher concentrations to generate a series of defined proportions. These mixtures were then measured using the Nu.Q® H3.1 ELISA (A) or ChLIA (B). Scatter plots display the linearity of the measurements, expressed in ng/mL, across the expected concentration intervals. The red line represents the linear regression fit.

A

ELISA					
Sample	Mean (ng/mL)	Within-run precision		Total precision	
		SD	%CV	SD	%CV
S1	15.1	1.8	11.8	2.9	19.3
S2	33.6	2.3	6.8	4.1	12.1
S3	106.5	7.3	7.1	9.8	9.2
S4	236.4	17.3	7.3	23.8	10.1
S5	298.8	12.7	4.2	25.5	8,5
S6	487.3	40.2	8.3	55.7	11.4

B

ChLIA					
Sample	Mean (ng/mL)	Within-run precision		Total precision	
		SD	%CV	SD	%CV
S1	15.2	0.4	2.7	1.1	7.4
S2	53.2	1.0	1.8	3.3	6.3
S3	210.1	2.9	1.4	14.7	7.0
S4	442.8	6.6	1.5	37.9	8.6
S5	735.9	16.5	2.2	67.7	9.2

Figure 4: Precision of the Nu.Q® H3.1 immunassays

Within-run precision (intra-assay) and total precision (inter-assay) for the ELISA platform (A) and the ChLIA platform (B). SD : Standard deviation; CV: Coefficient of variation.

The Nu.Q® H3.1 ChLIA is an automated chemiluminescent immunoassay using magnetic particles coated with an anti-histone H3.1 capture antibody and a detection antibody labeled with acridinium ester (Fig. 1B). This platform, analytically validated on K2EDTA plasma, exhibited enhanced analytical sensitivity, with a LOB of 0.3 ng/mL, a LOD of 0.9 ng/mL, and a LOQ of 2.9 ng/mL (Fig. 2). The assay showed linearity from 26.3 to 1214.4 ng/mL, which could be extended up to 6,000 ng/mL through a validated 5-fold automatic dilution for high-concentration samples (Fig. 3B). Precision was excellent, with intra-assay CVs ranging from 1.4% to 2.7% and total precision from 6.3% to 9.2%, indicating high consistency and reliability (Fig. 4B). The ChLIA format enables high-throughput testing and full automation, making it particularly suitable for clinical laboratories and large-scale routine analysis. Its wide dynamic range further supports accurate detection of varying levels of cell death, while also making the platform well suited for research laboratory applications.

Nu.Q® H3.1 was analytically validated in human K2EDTA plasma, with demonstrated compatibility across multiple sample types and animal species. (Van den Ackerveken, P., et al. 2025 J. Biol. Chem.)

Mono- and oligo-nucleosomes measurements: a key differentiating feature

The Nu.Q® H3.1 immunoassays enable the detection of both mono- and oligo-nucleosomes, thereby capturing nucleosome fragments generated from multiple cell death pathways. This capability is supported by the immunoassay results, which demonstrate detection of circulating mononucleosomes with different DNA fragment sizes ranging from 147 base pairs (bp) to 187 bp, as well as larger oligo-nucleosome structures (Fig 5)

Together, these results demonstrate that the assay captures a broad spectrum of nucleosome-associated DNA fragments, enabling comprehensive monitoring of nucleosome release associated with different mechanism of cell death.

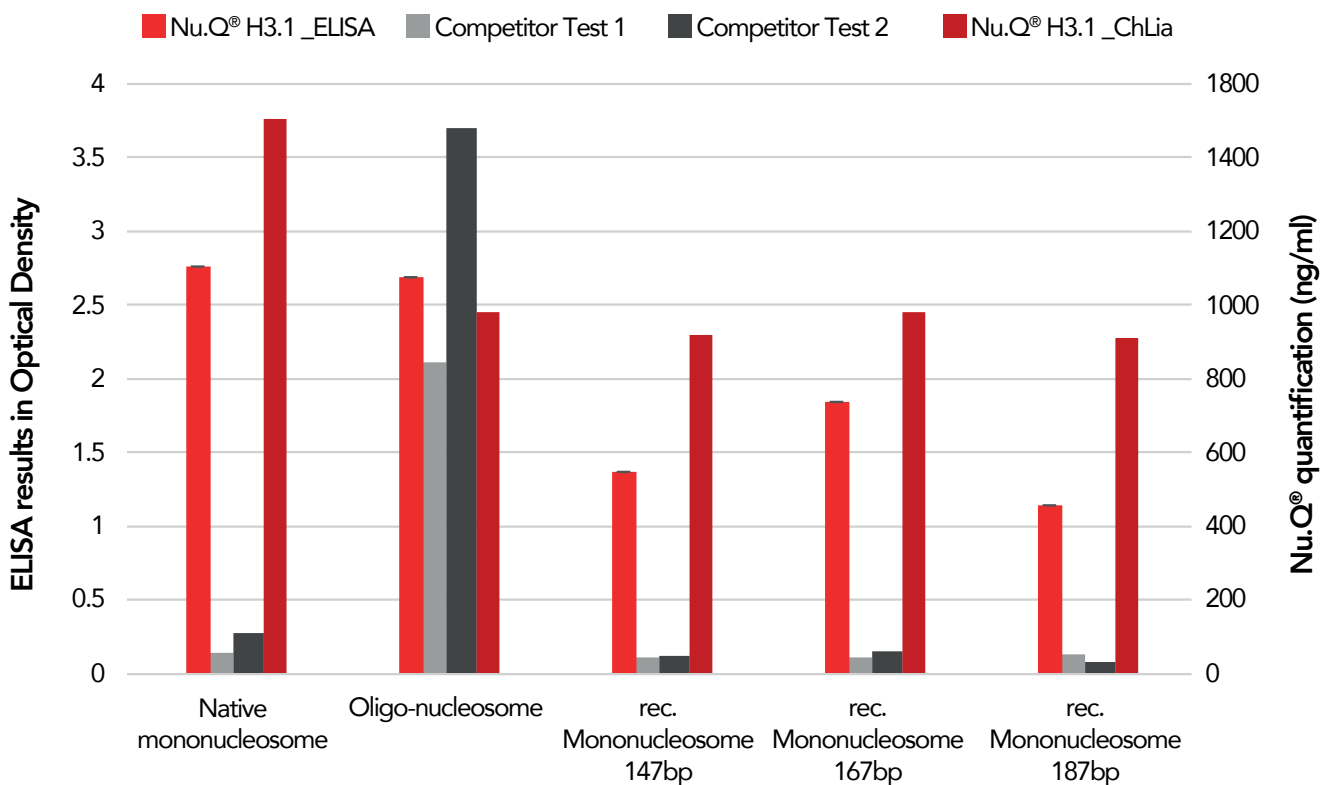


Figure 5: Nu.Q® H3.1 immunoassays detect both mono- and oligo-nucleosomes.

The histograms show the levels of various nucleosome types - mononucleosome, oligo-nucleosome or recombinant nucleosomes with different DNA lengths - measured using the Nu.Q® H3.1 ELISA (red bars), Nu.Q® H3.1 ChLIA (dark red bars), and two Cell Death competitor ELISA assays (light and dark grey bars). Results are expressed as optical density (OD; left scale) to enable comparison across ELISA assays and in ng/mL for the Nu.Q® assays (right scale).

A key differentiating feature of the Nu.Q® H3.1 assay is its ability to detect a broad spectrum of circulating nucleosomes, including mononucleosomes and larger oligo-nucleosomal complexes. Mononucleosomes, characterized by DNA fragments of approximately 147–187 bp, are a hallmark of apoptotic DNA fragmentation, while oligo-nucleosomes are associated with more complex nucleosomal patterns arising from various

Cell Death Monitoring in Action

cell death processes. As illustrated in the comparative analysis (Fig. 5), the Nu.Q® H3.1 assay efficiently detects these smaller nucleosome fragments, whereas the comparator assays show very limited sensitivity for this nucleosome population. This difference likely reflects variations in assay design and epitope accessibility, which can influence the detection of nucleosomes with distinct structural configurations.

By enabling the quantification of both mono- and oligo-nucleosomes, the Nu.Q® H3.1 assay provides a more comprehensive assessment of circulating nucleosomes profiles. This represents a significant advantage, as it enhances the ability to monitor apoptotic as well as broader cell death mechanisms.

Case study 1:

Monitoring of staurosporine-induced apoptosis in HeLa cells

HeLa cells were treated with 1 μ M staurosporine, an established apoptosis inducer, for 0h, 2h, 6h or 24h. The caspase-mediated PARP-1 (Poly(ADP-ribose) Polymerase-1) cleavage, a well-established marker of apoptosis, was first evaluated by Western blot. The abundance of the cleaved form of the protein (89kDa) clearly increases after 6h and 24h of treatment, confirming the activation of caspases. In parallel, cytoplasm fractions of the HeLa cells treated with staurosporine were prepared and measured using both the Nu.Q® H3.1 ELISA and ChLIA. Results, expressed as fold changes compared with the untreated HeLa cells, clearly show an increase in nucleosome concentrations with the duration of treatment, already detected after 2h. These interesting results demonstrate that the Nu.Q® H3.1 ELISA and ChLIA can be used as a sensitive and quantitative method to monitor apoptosis in culture cells.

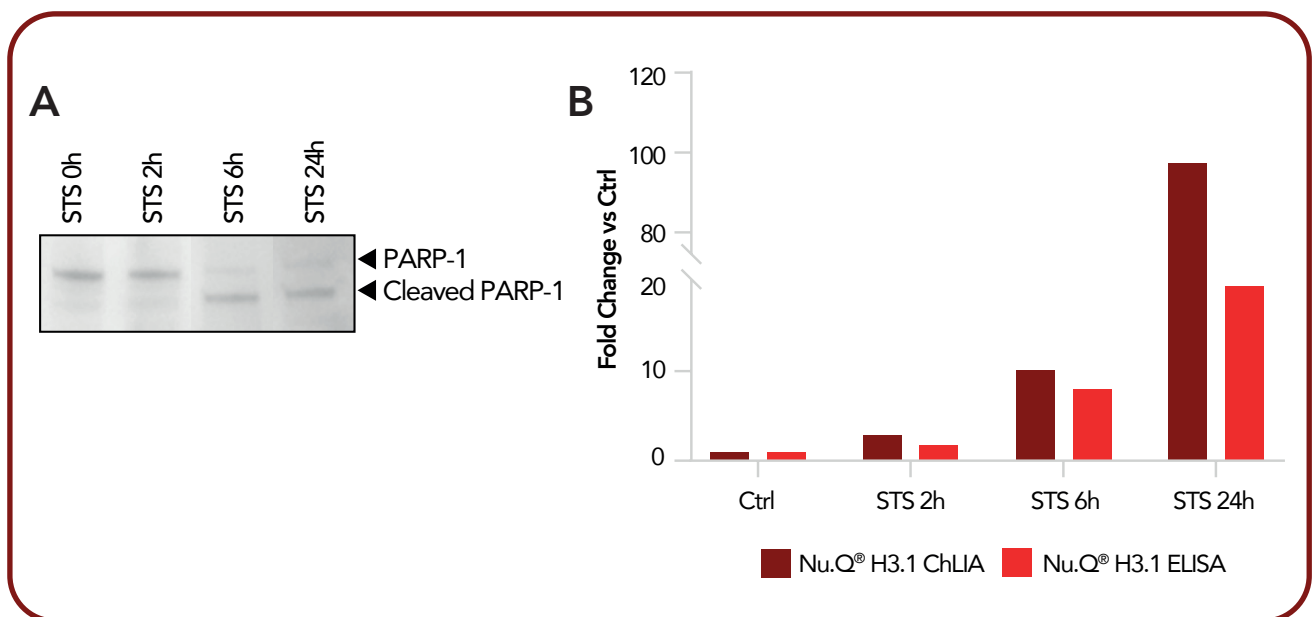


Figure 6: Detection of staurosporine induced-apoptosis in HeLa cells.

HeLa cells were treated with 1 μ M staurosporine (STS) for 0h, 2h, 6h or 24h. A) Total cell lysates were prepared and the caspase-induced PARP-1 cleavage was evaluated by Western blot as an indicator of apoptosis. B) Cytoplasmic protein extracts were prepared and tested with the Nu.Q® H3.1 ChLIA (back bars) or the Nu.Q® H3.1 ELISA (red bars). Nucleosomes concentrations were normalized by the protein content, and results are expressed as fold changed compared with the untreated condition (Ctrl = 1).

Case study 2:

NETosis induction in differentiated HL-60 cells.

NETosis induction in differentiated HL-60 cells resulted in a time-dependent increase in circulating nucleosome levels, as measured by the Nu.Q® H3.1 ChLIA assay. The observed elevation following PMA stimulation compared to untreated controls demonstrates the ability of the assay to detect nucleosome release associated with NET formation. These results further highlight the potential of the Nu.Q® H3.1 assay as a universal tool for monitoring nucleosome release across multiple cell death pathways.

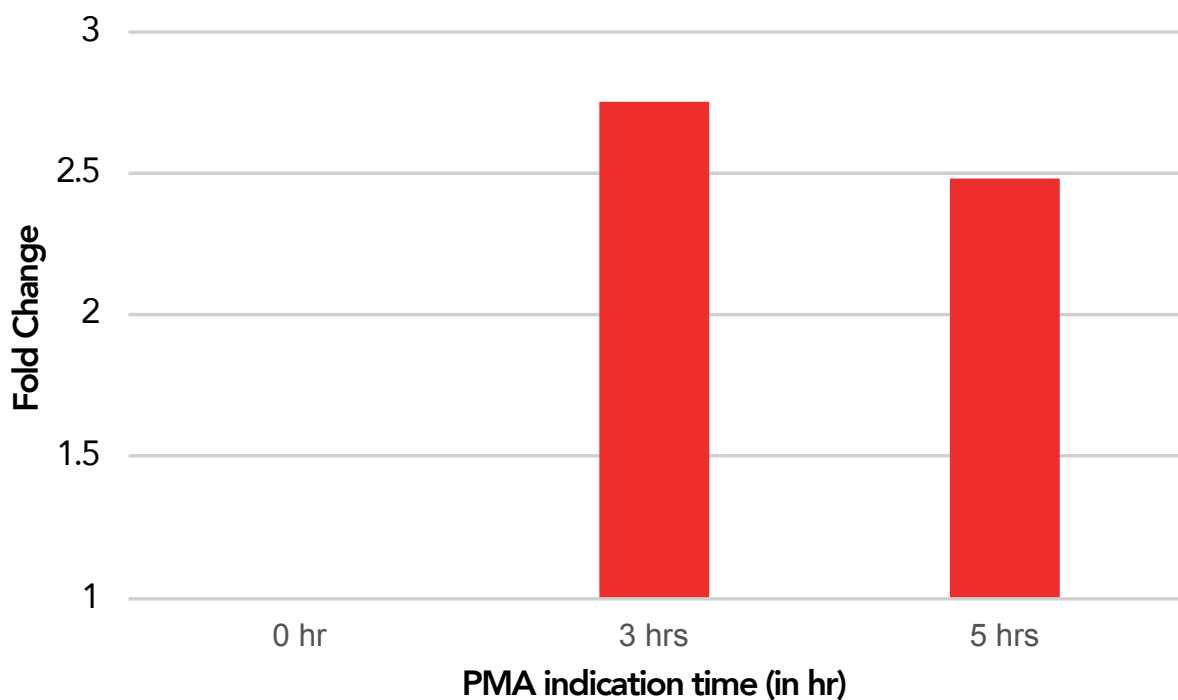


Figure 7: Evaluation of NETs release after NETosis induction in HL-60 cells.

HL-60 promyeloblastic cells were differentiated into neutrophil-like cells by treatment with 1.5% dimethyl sulfoxide (DMSO) during 6 days. NETosis was subsequently induced using 100nM phorbol 12-myristate 13-acetate (PMA), and two post-treatment time points were assessed. NET release was quantified using the Nu.Q® H3.1 ChLIA assay. For each time point, a PMA/untreated ratio was calculated, and results are expressed as fold change relative to the 0-hour control condition.

Case study 3: Monitoring Cell Death in Cell culture.

Human dermal microvascular endothelial cells (HMEC-1) cells were cultured for 12 hours before the growth medium was replaced (Ungerer et al., Sci Rep, 2021). From this starting point, flasks were incubated for 4, 8, 12, 24, 36, 56, and 80 h without changing the medium. At each time point, the culture supernatant was collected and 50ul were evaluated with Nu.Q® ChLIA immunoassay. Increasing incubation times were associated with active and passive cell death events. Correspondingly, nucleosome concentrations in the supernatant increased over time, reflecting the progression of cell death events.

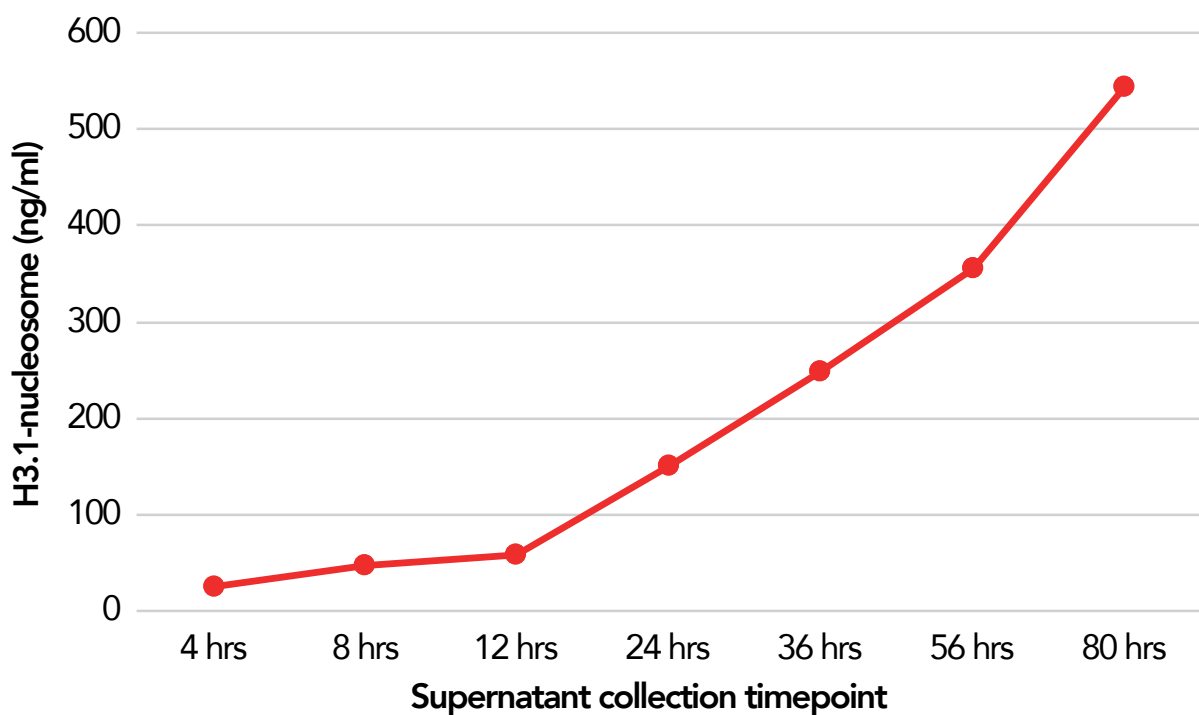


Figure 8: Cell death detection in cell culture supernatant.

HMEC-1 cells were incubated for up to 80 hours without changing the cell culture medium, and nucleosome concentrations in 50ul of cell culture supernatants were measured at each time point using the Nu.Q® H3.1 ChLIA immunoassay.



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