Cell Death Detection ELISA^{PLUS}

Cat. No. 11 774 425 001 96 tests

Cat. No. 11 920 685 001 10 x 96 tests

Type	One-step sandwich ELISA, colorimetric
Useful for	Relative quantification of apoptosis without cell labeling; differentiating apoptosis from necrosis
Samples	Cell lysates, cell culture supernatants, serum, or plasma
Method	Cell lysis, followed by immunochemical determination of histone-complexed DNA fragments in a microplate well ***(Note:** For detection of necrosis, histone-complexed DNA fragments are detected directly in the culture supernatant, without cell lysis)*
Time	Approx. 3 h (after induction of apoptosis)

Significance of kit: Use this kit for relative quantification of histone-complexed DNA fragments (mono- and oligonucleosomes) out of the cytoplasm of cells after the induction of apoptosis or when released from necrotic cells. Since the assay does not require prelabeling of cells, it can detect internucleosomal degradation of genomic DNA during apoptosis even in cells that do not proliferate *in vitro* (for example, freshly isolated tumor cells). The antibodies used in the assay are not species-specific, so the kit may be used to assay cells from a wide variety of species (see "Other applications" in this article).

Test principle: The assay uses an one-step sandwich immunoassay to detect nucleosomes. The procedure (Figure 7 and Flow Chart 2) involves:

1	Incubating cells in a microplate well (for instance, 10^4 human cells in 200 µl culture) with an agent that induces cell death (for example, campothecin (CAM)). After the incubation, the cells are pelleted by centrifugation and the supernatant is (containing DNA from necrotic cells that leaked through the membrane during incubation) discarded.
2	Resuspending and incubating cells in lysis buffer. After lysis, intact nuclei are pelleted by centrifugation.
3	Transferring an aliquot of the supernatant to a streptavidin-coated well of a microplate.
4	Binding nucleosomes in the supernatant with two monoclonal antibodies, anti-histone (biotin-labeled) and anti-DNA (peroxidase-conjugated). Antibody-nucleosome complexes are bound to the microplate by the streptavidin.
5	Washing the immobilized antibody-histone complexes three times to remove cell components that are not immunoreactive.
6	Incubating sample with peroxidase substrate (ABTS).
7	Determining the amount of colored product (and thus, of immobilized antibody-histone complexes) spectrophotometrically.

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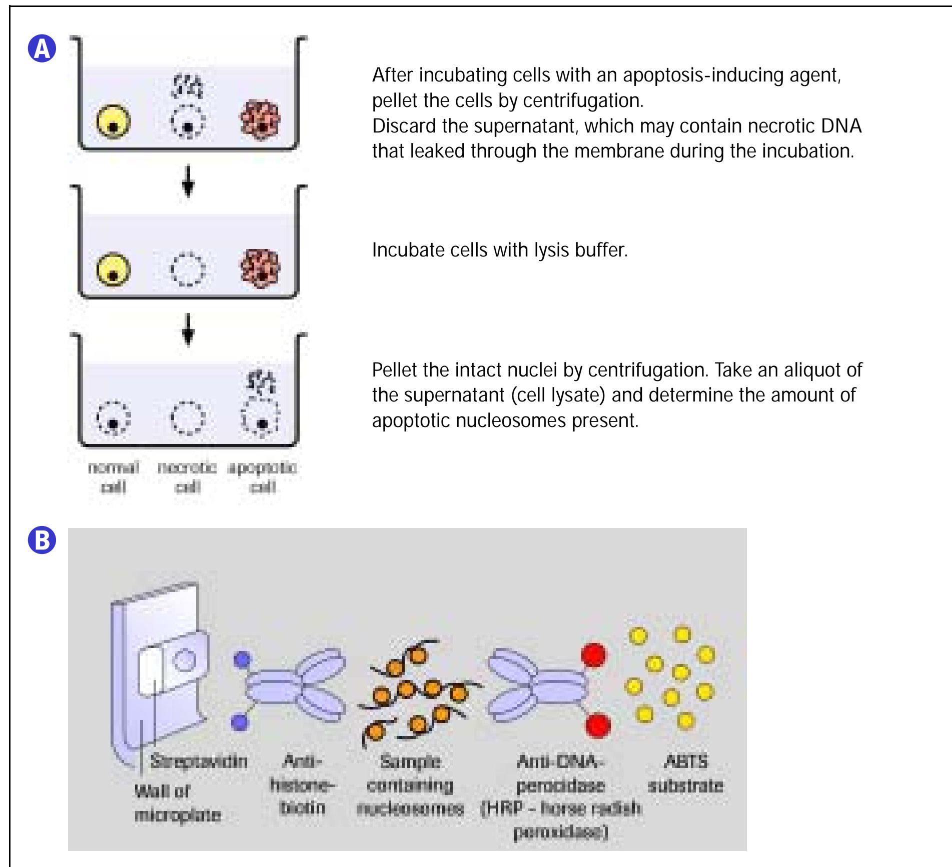
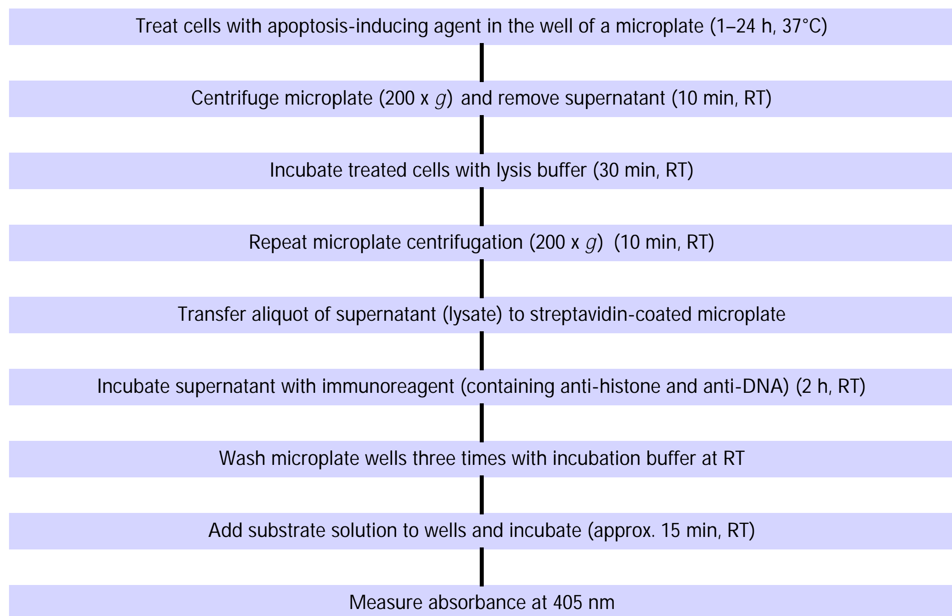
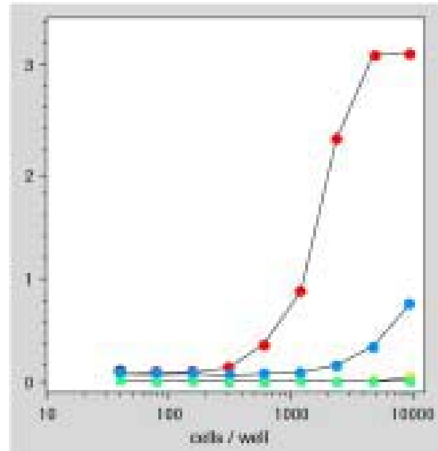


▲ **Figure 7: How the Cell Death Detection ELISA^{PLUS} works.**
Panel A: Sample preparation
Panel B: ELISA



▲ **Flow Chart 2: Assay procedure, Cell Death Detection ELISA^{PLUS}.**

Sensitivity: In a model system, nucleosomes were detectable in as few as 600 campothecin-induced U937 cells (Figure 8). However, the lower limit for detecting dying/dead cells in a particular sample varies with the kinetics of the apoptotic process, the cytotoxic agent used, and the number of affected cells in the total cell population.



◀ **Figure 8: Sensitivity of Cell Death Detection ELISA^{PLUS}.**
Different cell concentrations of U937 cells were incubated with camptothecin (CAM) (2 µg/ml) or without CAM for 4 h at 37°C. 20 µl of cell culture supernatant and cell lysates were analyzed in the ELISA. Substrate reaction time: 10 min. ● Lysate with CAM, ● Lysate without CAM, ■ Supernatant with CAM, ■ Supernatant without CAM.
Result: The ELISA can clearly detect apoptosis-related nucleosomes in as few as 600 cells.

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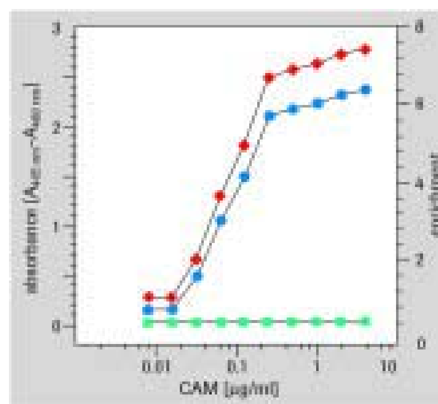
Specificity: The ELISA is specific for nucleosomes containing single- or double-stranded DNA (Figure 9). It is not species specific.



◀ **Figure 9: Dose-response experiment analyzed by the Cell Death Detection ELISA^{PLUS}.**
U937 cells (10^4 cells/well, in 200 µl) were incubated with different concentrations of camptothecin (CAM) for 4 h at 37°C. Before and after lysis, cells were centrifuged and a 20 µl aliquot of the supernatant was analyzed with the Cell Death Detection ELISA^{PUS}. Results were plotted as dose vs. response. Substrate reaction time: 5 min. ◆ Lysate, ■ Supernatant, ● Enrichment factor of the lysate.
Result: Amounts of cytoplasmic oligonucleosomes (an indicator of apoptosis) increase as CAM concentration increases. Cell culture supernatants removed from the cells after treatment (but before lysis) gave no signal, indicating that there are no necrotic cells during the treatment.

Can be used to assay:

- Adherent cells
- Cells in suspension culture
- Cell culture supernatant
- Lysates of cells obtained *ex vivo*
- Serum, or plasma

Kit contents

1. Anti-histone antibody (clone H11-4), biotin-labeled
2. Anti-DNA antibody (clone M-CA-33), peroxidase-conjugated
3. DNA-histone complex (positive control)
4. Incubation buffer, ready-to-use
5. Lysis buffer, ready-to-use
6. Substrate buffer, ready-to-use
7. ABTS substrate tablets
8. Microplate modules (12 x 8 wells)
9. Adhesive plate cover

Typical results: see Figure 8 and 9

Other applications: For more examples of how the Cell Death Detection ELISA^{PLUS} and the Cell Death Detection ELISA can be used in the lab, see Appendix, pages 129–131.