

Cellular DNA Fragmentation ELISA

Cat. No. 11 585 045 001 500 tests

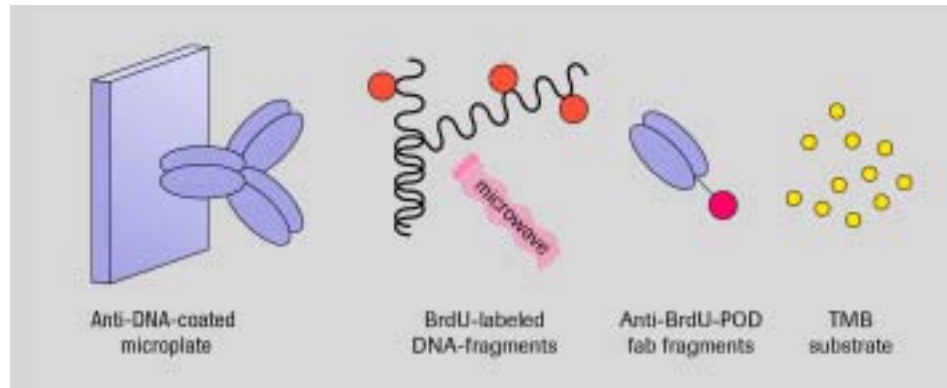
Type	Sandwich ELISA, colorimetric
Useful for	Quantitation of BrdU-labeled DNA fragments either released from cells during necrosis or cell-mediated cytotoxicity, or within the cytoplasm of apoptotic cells
Samples	Cell-free supernatants from cultured cells or cytoplasmic lysates of cells, prelabeled with BrdU
Method	Prelabeling of cells with BrdU, followed by immunodetection of BrdU-labeled DNA fragments in sample
Time	4.5–5.5 h (+ BrdU labeling and induction of cell death)

Significance of kit: The Cellular DNA Fragmentation ELISA measures apoptosis, necrosis, or cell mediated cytotoxicity by quantitating the fragmentation and/or release of BrdU-labeled DNA. The kit detects DNA fragments:

- In the cytoplasm of apoptotic cells, thus providing a non-radioactive alternative to the [³H]-thymidine-based DNA fragmentation assay.
- Released into the culture supernatant during cell mediated cytotoxicity, thus providing a non-radioactive alternative to the [³H]-thymidine- and [⁵¹Cr]-release assays.

Test principle: The assay is a sandwich enzyme-linked immunosorbent assay (ELISA). It uses two mouse monoclonal antibodies: one directed against DNA the other against BrdU (Figure 46). The procedure (Flow Chart 13) involves:

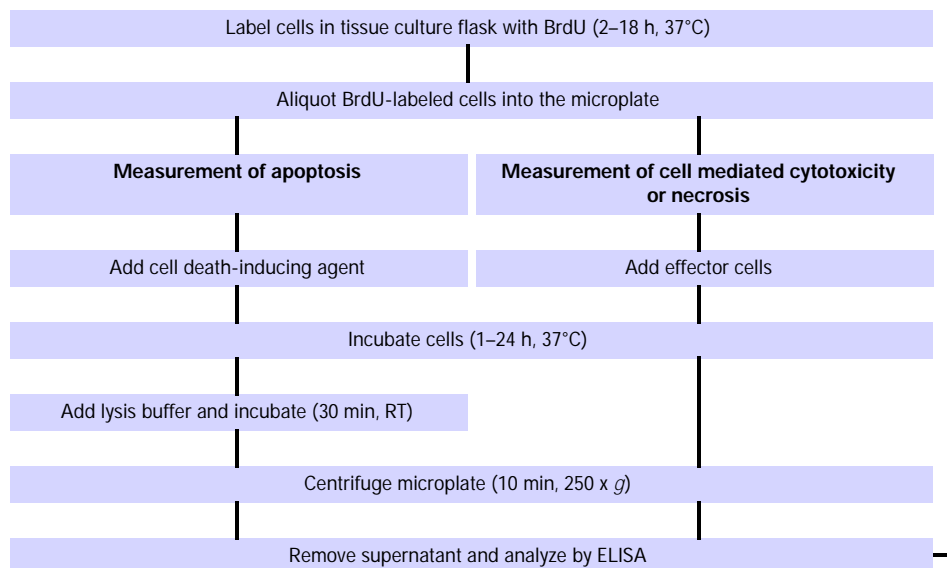
- 1 Prelabeling of cells with BrdU.
- 2 Incubating the labeled cells in the presence of either an apoptosis inducing agent or effector cells (for cell mediated cytotoxicity). At the end of the incubation, cells are centrifuged and either supernatant is analyzed (for cell mediated cytotoxicity or necrosis) or cellular lysate is analyzed for apoptosis. The supernatant, containing LMW-DNA is used for the assay. If desired, both sample types can be prepared and assayed (see Flow Chart 13).
- 3 Adsorbing the Anti-DNA antibody onto the wells of a microplate.
- 4 Adding the supernatant of Step 2 to the microplate. BrdU-labeled DNA fragments in the sample bind to the immobilized Anti-DNA antibody.
- 5 Denaturing the immunocomplexed BrdU-labeled DNA-fragments by microwave irradiation or nuclease treatment. This procedure is necessary for the accessibility of the BrdU antigen.
- 6 Reacting Anti-BrdU antibody peroxidase conjugate (Anti-BrdU-POD) with the BrdU-labeled DNA to form an immunocomplex.
- 7 Quantitating the bound Anti-BrdU-POD in the immunocomplex with a peroxidase substrate (TMB).



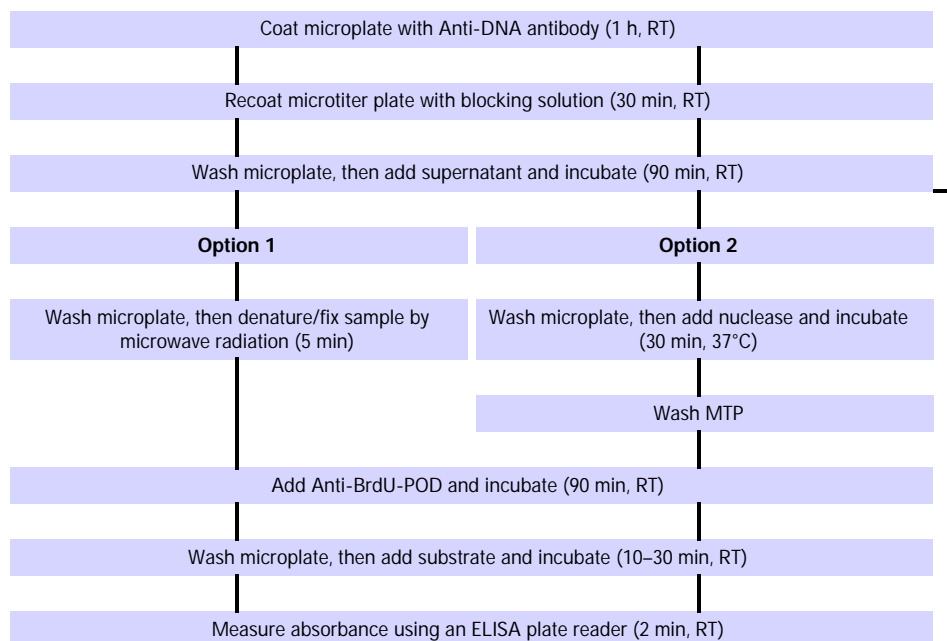
▲ Figure 46: How the Cellular DNA Fragmentation ELISA works.

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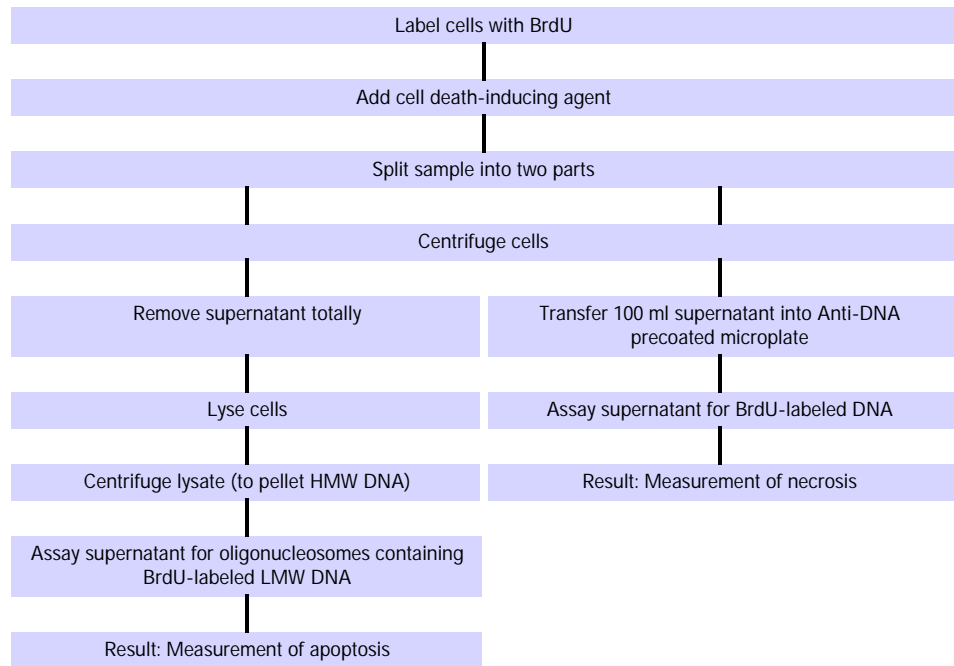
Sample preparation:



ELISA



▲ Flow Chart 13: Assay procedure, Cellular DNA Fragmentation ELISA.



▲ Flow Chart 14: Simultaneous analysis of apoptosis and necrosis in the same sample with the Cellular DNA Fragmentation ELISA.

Sensitivity

Apoptosis: When HL60/CAM is used as a model system for apoptosis, the ELISA can detect BrdU-labeled DNA fragments in the cytoplasm of 1×10^3 cells/well (Figure 42).

Cell mediated cytotoxicity: When allogeneic-stimulated cytotoxic T cells are used as effector cells to lyse P815 target cells in a cell mediated cytotoxicity assay, the ELISA can detect BrdU-labeled DNA fragments from 2×10^3 target cells/well.

Note: The ability to detect a minimum number of dying/dead cells in a particular sample strongly depends on the kinetics of cell death, the cytotoxic agent or the effector cells used to induce cell death, and the amount of BrdU incorporated into the target cells.

Specificity

- ▶ The Anti-DNA antibody binds to single- and double-stranded DNA. It shows no cross-reactivity with BrdU.
- ▶ The conjugated antibody (Anti-BrdU-POD, Fab fragments) will bind to BrdU-labeled DNA after the DNA is partially denatured. The antibody specifically recognizes 5-bromo-2'-deoxy-uridine. The antibody conjugate shows no cross-reactivity with any endogenous cellular components such as thymidine or uridine.
- ▶ The ELISA specifically detects BrdU-labeled DNA fragments in culture supernatant and cytoplasm. The ELISA can detect BrdU-labeled DNA from any species, so the assay is not species-restricted.

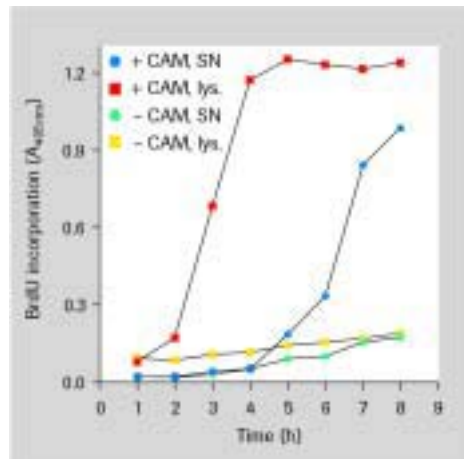
Can be used to assay:

- Culture supernatant and cytoplasmic fractions (lysates) of cells whose DNA have been metabolically pre-labeled with BrdU (*e.g.*, cell lines and other *in vitro* proliferating cells). Thus, only cells which proliferate *in vitro* can be used.

Kit contents

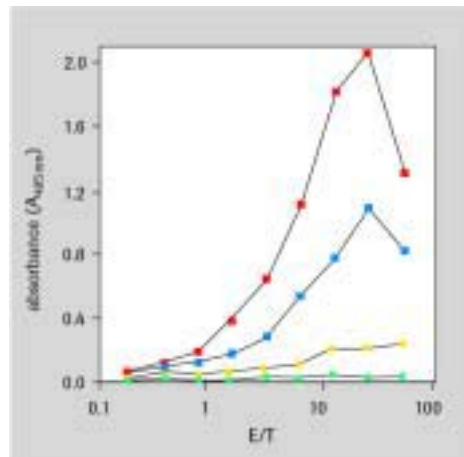
1. Anti-DNA antibody (clone M-CA-33)
2. Anti-BrdU-POD, Fab fragments (clone BMG 6H8)
3. Coating buffer
4. Washing buffer
5. Incubation buffer
6. Substrate solution
7. BrdU labeling reagent
8. Adhesive cover foils

Typical results: see Figures 47 and 48.



▲ **Figure 47: Kinetics of camptothecin (CAM) induced cell death in HL60 cells.** Cells were prelabeled with BrdU overnight. Then, cells (1×10^4 /well) were incubated either in the presence of 200 ng/ml CAM (●, ■) or without CAM (○, □) for 1–8 h. Supernatant (100 μ l/well) was removed, then cells were lysed and both supernatant (●, ○) and lysate (■, □) were analyzed by Cellular DNA Fragmentation ELISA.

Result: Apoptosis clearly occurs after 3–4 h incubation. After 6–8 h, secondary necrosis begins to be seen.



▲ **Figure 48: Kinetics of cytotoxic T lymphocyte (CTL)-mediated cytotoxicity in P815 target cells quantified with the Cellular DNA Fragmentation ELISA.** 2×10^4 BrdU-labeled target cells/well were incubated with CTLs at different effector-to-target ratios (E/T) for varying times. After incubation for 1 h (●), 2 h (○), 4 h (■), and 6 h (□), culture supernatant samples (100 μ l/well) were assayed for DNA fragments.

Other applications: For more examples of how the Cellular DNA Fragmentation ELISA can be used in the lab, see Appendix, page 143.