

Cytotoxicity Detection Kit (LDH)

Cat. No. 11 644 793 001 2000 tests

Type	Colorimetric assay, microplate format
Useful for	Quantitation of LDH activity released from damaged/dying cells
Samples	Cell-free supernatants from cells in culture
Method	Preparation of cell-free supernatant, followed by incubation of supernatant with INT to form colored formazan, a product which may be quantitated colorimetrically
Time	0.5–1 h (+ induction of cell death)

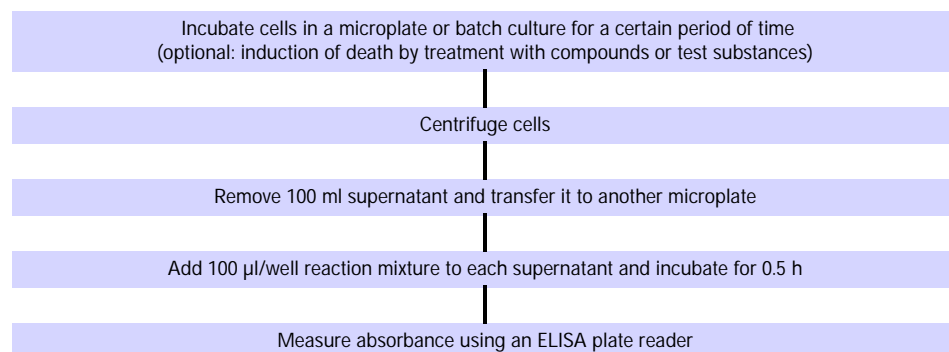
Significance of kit: The Cytotoxicity Detection Kit measures cytotoxicity and cell lysis by detecting LDH activity released from damaged cells. The assay is performed in a 96-well microplate. The kit can be used in many different *in vitro* cell systems where damage of the plasma membrane occurs. Examples are:

- Detection and quantification of cell mediated cytotoxicity.
- Determination of mediator-induced cytolysis.
- Determination of the cytotoxic potential of compounds in environmental and medical research, and in the food, cosmetic, and pharmaceutical industries.
- Determination of cell death in bioreactors.

Test principle: The assay is based on the cleavage of a tetrazolium salt when LDH is present in the culture supernatant. The procedure involves:

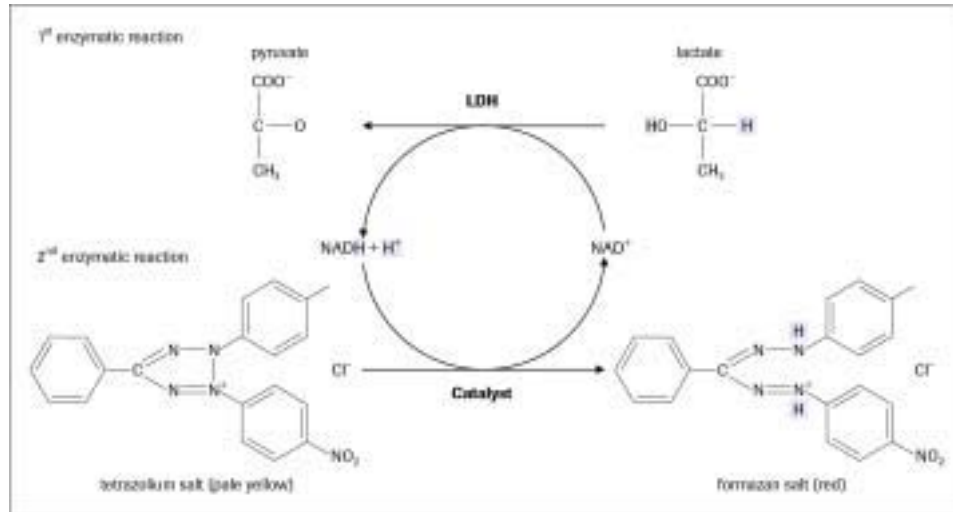
- 1 Incubating the cells in culture to allow cell death to occur. An increase in the amount of dead or plasma membrane-damaged cells during the assay results in an increase of LDH in the culture supernatant.
- 2 Collecting the cell-free culture supernatant.
- 3 Adding the substrate mixture from the kit to the culture supernatant. Any LDH released into the supernatant during Step 1 will reduce the tetrazolium salt INT to formazan by a coupled enzymatic reaction. Thus, release of LDH into the supernatant directly correlates to the amount of formazan formed in this step.
- 4 Quantitating the formazan dye formed in an ELISA plate reader. The formazan dye formed is water-soluble and shows a broad absorption maximum at about 500 nm.

For a detailed overview of the steps involved in the procedure, see Figures 42 and 43 and Flow Chart 12.



▲ Flow Chart 12: Assay procedure, Cytotoxicity Detection Kit (LDH).

A



▲ **Figure 43: Biochemistry of the Cyto-toxicity Detection Kit (LDH):** In the first enzymatic reaction LDH reduces NAD⁺ to NADH + H⁺ by oxidation of lactate to pyruvate; in the second enzymatic reaction the catalyst (diaphorase) transfers H⁺ from NADH + H⁺ to the tetrazolium salt INT.

Can be used to assay:

- Cell-free supernatants obtained from cells cultured in 96-well microplates or batch cultures.

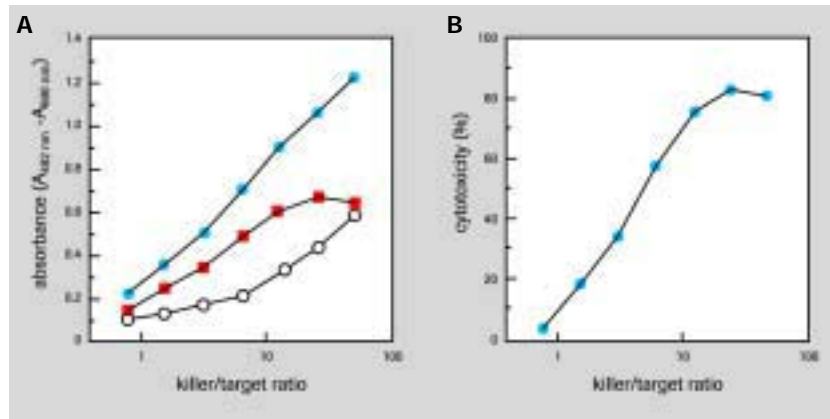
Kit contents

1. Catalyst (Diaphorase/NAD + mixture)
2. Dye solution (INT and sodium lactate)

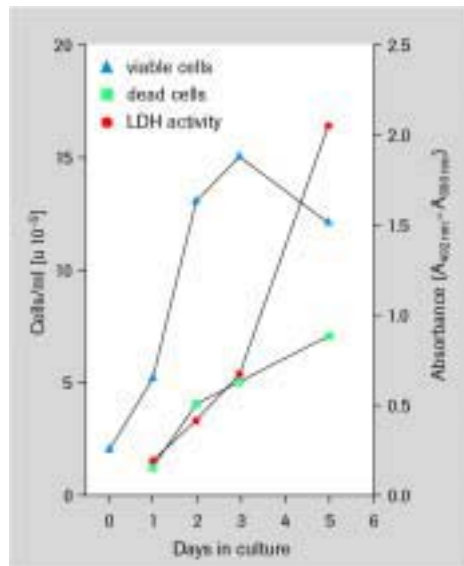
Note: To prepare the reaction mixture, mix catalyst with dye solution prior to use.

Typical results: see Figures 44 and 45.

Other applications: For more examples of how the Cytotoxicity Detection Kit (LDH) can be used in the lab, see Appendix, pages 142–143.



▲ **Figure 44. Determination of the cytolytic activity of allogen-stimulated cytotoxic T lymphocytes (CTLs) using the Cytotoxicity Detection Kit (LDH).** A) Spleen cells of C57/Bl 6 mice (H-2b) were stimulated *in vitro* with P815 cells (H-2d). Viable CTLs were purified and titrated in the microplate as described in the package insert. Target cells (1×10^4 cells/well) were incubated in the presence or absence (effector cell controls) of effector cells for 4 hours. Culture supernatant samples (100 μ l/well) for effector controls (○) and the effector-target cell mix (●) were assayed for LDH activity. The middle curve is generated when the background control values are subtracted from the effector-target cell values (■). B) Calculated percentage of cell-mediated lysis.



▲ **Figure 45: Correlation of cell death (defined by increased plasma membrane permeability) and LDH release.** Ag8 cells (starting cell concentration: 2×10^5 /ml) were cultured and after 1, 2, 3 and 5 days, aliquots were removed. The amount of viable (▲) and dead (■) cells was determined by trypan blue exclusion. LDH activity in cell free culture supernatant was determined using the Cytotoxicity Detection Kit (●).

Result: Increased LDH release clearly correlated with the increase of dead cells.