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Product Information

***In Vitro* Toxicology Assay Kit,**
Lactic Dehydrogenase based

Catalog Number **TOX7**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Traditionally, the toxic effects of unknown compounds have been measured *in vitro* by counting viable cells after staining with a vital dye. Alternative methods include the measurement of DNA synthesis by radioisotope incorporation, cell counting by automated counters, and other methods that rely on dyes and cellular activity. The lactate dehydrogenase assay is a means of measuring either the number of cells via total cytoplasmic lactate dehydrogenase (LDH) or membrane integrity as a function of the amount of cytoplasmic LDH released into the medium.

The lactate dehydrogenase method is simple, accurate and yields reproducible results. The assay is based on the reduction of NAD by LDH. The resulting reduced NAD (NADH) is utilized in the stoichiometric conversion of a tetrazolium dye. The resulting colored compound is measured spectrophotometrically. If the cells are lysed prior to assaying the medium, an increase or decrease in cell numbers results in a concomitant change in the amount of substrate converted. This indicates the degree of inhibition of cell growth (cytotoxicity) caused by the test material. If cell-free aliquots of the medium from cultures given different treatments are assayed, then the amount of LDH activity can be used as an indicator of relative cell viability as well as a function of membrane integrity. This technique has been utilized as an alternative to ^{51}Cr release for cell mediated cytotoxicity assays,[1] as well as conventional cytotoxicity resulting from interaction of a test material with the cell.[2]

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

It is recommended the entire procedure be reviewed before starting the assay.

Components

Catalog Number	Item	Quantity
L2402	LDH Assay Substrate Solution	25 ml
L2527	LDH Assay Cofactor Preparation	25 ml
L2277	LDH Assay Dye Solution (see note below)	25 ml
L2152	LDH Assay Lysis Solution	10 ml

Note: The LDH Assay Dye Solution (Catalog Number L2277) is a saturated solution and some precipitation may appear upon thawing. The product will partially dissolve when warmed to 37 °C, the resulting solution can be decanted away from any remaining precipitate. Precipitated dye added to reactions or removed from the solution will not affect the performance or results obtained from this kit.

Material Required but Not Provided

1 N Hydrochloric Acid, (HCl, Catalog Number H9892)

Preparation Instructions

Prepare 25 ml of 1× LDH Assay Cofactor Preparation by adding 25 ml of tissue culture grade water to the bottle of lyophilized cofactors (Catalog Number L2527). The 1× LDH Assay Cofactor Preparation is stable when stored at –20 °C (avoid frost-free freezers) in working aliquots to avoid repeated freeze/thaw procedures. Storage at 2–8 °C may result in loss of activity and yield erroneous results.

Storage/Stability

All kit components are stable when stored at –20 °C. Product label bears expiration date.

Procedure

The lactate dehydrogenase method of monitoring *in vitro* cytotoxicity is well suited to multiwell plates. For best results, cells in the log phase of growth should be employed and the final cell number should not exceed 10^6 cells/cm^2. Each test should include a blank containing complete medium without cells.

Notes: Uneven evaporation of culture fluid in wells of multiwell plates may cause erroneous results.

NADH is subject to photodegradation. Excessive exposure of the reaction to light can reduce sensitivity and accuracy.

Serum contains a significant level of LDH activity. Use of medium containing serum can result in higher levels of LDH activity, sufficient to significantly decrease the sensitivity of assay and/or obscure detection of cellular LDH completely. It is recommended that serum-supplemented medium be replaced with medium without serum or medium supplemented with heat-inactivated serum.

Lactate dehydrogenase activity can be assayed by either of two methods. Method 1 provides a measure of total cell number. Method 2 assesses the membrane integrity of cells as a function of the amount of LDH leakage into the medium.

Method 1 - (Total LDH)

1. Remove cultures from incubator into a laminar flow hood or other sterile work area.
2. Add 1/10 volume of LDH Assay Lysis Solution (Catalog Number L2152) per well and return plate to incubator for 45 minutes.
3. Centrifuge plate at 250 × *g* for 4 minutes to pellet debris.
4. Transfer aliquot to clean flat-bottom plate and proceed with enzymatic analysis.

Method 2 - (LDH Release)

1. Remove cultures from incubator into a laminar flow hood or other sterile work area.
2. Centrifuge plate at 250 × *g* for 4 minutes to pellet cells.
3. Transfer aliquot to clean flat-bottom plate and proceed with enzymatic analysis.

Enzymatic Analysis

1. Prepare the Lactate Dehydrogenase Assay Mixture by mixing equal volumes of LDH Assay Substrate Solution (Catalog Number L2402), LDH Assay Dye Solution (Catalog Number L2277), and 1× LDH Assay Cofactor Preparation. Prepare the Lactate Dehydrogenase Assay Mixture at time of use, extended storage of the assay mixture is not recommended.
2. Remove an aliquot of the medium for testing (approximately half of the volume of the culture medium). Add the Lactate Dehydrogenase Assay Mixture to each sample in a volume equal to twice the volume of medium removed for testing.
3. Cover the plate with an opaque material to protect from light (e.g., aluminum foil or a box) and incubate at room temperature for 20–30 minutes.
4. The reaction can be terminated by the addition of 1/10 volume of 1 N HCl to each well.
5. Spectrophotometrically measure absorbance at a wavelength of 490 nm. Measure the background absorbance of the multiwell plates at 690 nm and subtract this value from the primary wavelength measurement (490 nm).
6. Tests in multiwell plates can be read in a plate reader or the contents of individual wells can be transferred to appropriate sized cuvettes for spectrophotometric measurement.

References

1. Decker, T., and Lohmann-Matthes, M-L., A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. J. Immunol. Methods, **15**, 61-69 (1988).
2. Legrand, C. et al., Lactate dehydrogenase (LDH) activity of the number of dead cells in the medium of cultured eukaryotic cells as marker. J. Biotechnol., 25, 231-243 (1992).

JF,MAM 09/09-1