

IN CYTOTOX



ANIARA

Manufactured By: Xenometrix AG

PAN I:

LDHe-XTT-NR-SRB

APPLICATIONS

Combined colorimetric assays for the quantification of the membrane integrity, mitochondrial metabolism, lysosomal integrity and activity, and total protein synthesis rate of cells in response to pharmaceutical, chemical and environmental compounds, and nutrients.

PRINCIPLE

This kit allows to measure sequentially four cytotoxicity parameters in one single cell culture: Membrane integrity (**LDHe: Extracellular Lactate Dehydrogenase**), mitochondrial activity (**XTT: Tetrazolium Hydroxide**), lysosomal integrity and activity (**NR: Neutral Red**), and total protein synthesis rate (**SRB: Sulforhodamine B**).

Released LDH, a marker for cell damage, is determined kinetically in the medium (NADH consumption). XTT is reduced in the cells to formazan by mitochondrial succinate dehydrogenase. The reduction rate is measured and correlates with mitochondrial activity. The neutral red (NR) assay procedure is based on the ability of viable cells to incorporate and bind neutral red within lysosomes. The fixed SRB dye, measured photometrically after solubilization, correlates with total protein synthesis rate and therefore with cell proliferation.

Unlike some other LDHe assays, the In Cytotox LDHe assay measures the oxidation of NADH to NAD⁺ and the concurrent reduction of pyruvate to lactate. By providing an excess of pyruvate in the reaction mixture, the In Cytotox LDHe assay is therefore insensitive to pyruvate in the culture medium, which can cause product inhibition of the reverse reaction implemented in other LDHe assays.

BIOLOGICAL PARAMETERS EVALUATION

- IC₅₀ (Inhibitory Concentration 50%)
- Membrane integrity
- Respiratory chain activity
- Lysosomal activity
- Total protein synthesis
- Cell proliferation

TECHNICAL SPECIFICATIONS

- Absorbance:**
- LDHe: 340 nm
 - XTT: 480 (optimum) or 450 nm
 - NR: 540 nm
 - SRB: 540 nm
(Recommended reference filters: 690 nm)
- Approximate assay time (total):**
- 8 hr 30 + time to dry the plate (SRB) if LDH incubation is done in parallel with XTT incubation. SRB reading can also be done the next morning.
- Available kit configurations:**
- Reagents only
 - Reagents and 96-well microplates, sterile reagent reservoirs
- LDHe- XTT-NR-SRB Kit content:**
- Buffer solutions
 - Enzyme solution
 - Stop solution
 - Substrate solutions
 - Activator solution
 - Labeling solution
 - Wash solutions
 - Solubilization solutions
 - Rinsing solution
 - Fixing solutions
 - Instruction manual

PAN I: LDHe-XTT-NR-SRB-KIT SIZES AND REFERENCE NUMBERS

REFERENCE NO.	NUMBER OF TESTS
APAN I 96.300	4 x 300
APAN I 96.1200	4 x 1200

**Kits with plasticware (microplates and reservoirs) are also available.
Individual reagents and other kit sizes available upon request.**

Version 1.5 01/2007

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