



Manufactured By: Xenometrix AG

## 1. Introduction

The safety evaluation of compounds such as drugs, cosmetics, food additives, pesticides and industrial chemicals is growing year by year.

Cytotoxicity assays were among the first in vitro bioassay methods used to predict toxicity of substances to various tissues. They are widely used for the determination of cell proliferation, viability and activation. The need for reliable, sensitive and quantitative assays that would enable analysis of large number of tests in preclinical research is therefore increasing.

## 2. Objectives of the IN CYTOTOX Assays

Alternative methods to animal experimentation allow to limit the use of experimental test animals and to perform screening tests on a greater scale with much lower quantities of test compound. The alternative methods are the scientific and economic tool of choice for molecular or cellular high throughput screening.

Xenometrix offers with the IN CYTOTOX products a complete solution for the in vitro evaluation of tolerance, resistance and recovery of cells in response to pharmaceutical or chemical compounds. They are based on sensitive and fast high throughput methods.

IN CYTOTOX test kits can be used for any cell line as well as for spheroids resulting from human breast or colon cancer.

### a. Biological Parameters

The IN CYTOTOX high-throughput test systems allow the measurements of membrane integrity (LDHe), metabolic activity (GLU), respiratory chain activity (XTT/MTT), total protein synthesis (SRB), DNA content/cell number (CVDE), and lysosomal activity (PAC, NR). Studies of agonist and antagonist interactions can be performed.

The parameters ID50 or IC50 (50% inhibiting dose or concentration), NED (no effect dose) and IT50 (inhibiting time) can be determined.

### b. Combined Test Kits

The combined IN CYTOTOX test kits allow to determine up to four metabolic parameters from the same cellular sample. For example membrane integrity, cellular metabolism, mitochondrial activity and total protein synthesis or cell proliferation can be assessed from the same cells. This technical optimization used in the combined kits allows to increase the relevance of correlation of the tests, to reduce the handling time, and the amounts of test compound. It allows also to make optimal use of rare and valuable cell samples such as primary cell cultures.

### 3. Principle of the Assays

The IN CYTOTOX assays consist of four steps:

- trypsinization of the cells
- transfer to 96-well plates
- test compound incubation
- cytotoxicity assays

Cells are harvested by trypsinisation, counted, diluted and transferred to 96-well microtiter plates. (Non-adherent cells can be used as well but require more gentle handling and washing steps). After incubation for at least 24 hrs dilutions of test compound are added. The cells are exposed to the test compound for the desired length of time. Cell growth and viability are then measured by using several cytotoxicity assays.

The test results are measured spectrophotometrically at specific wave lengths. The analysis of the results can be performed using the CelTox software.

### 4. IN CYTOTOX Test Kits

#### One Parameter Kits

XTT and MTT Tetrazolium salt  
LDHe Extracellular Lactate Dehydrogenase  
NR Neutral Red  
PAC Acid Phosphatase  
SRB Sulforhodamine B  
GLU Glucose  
CVDE Crystal Violet Dye Elution

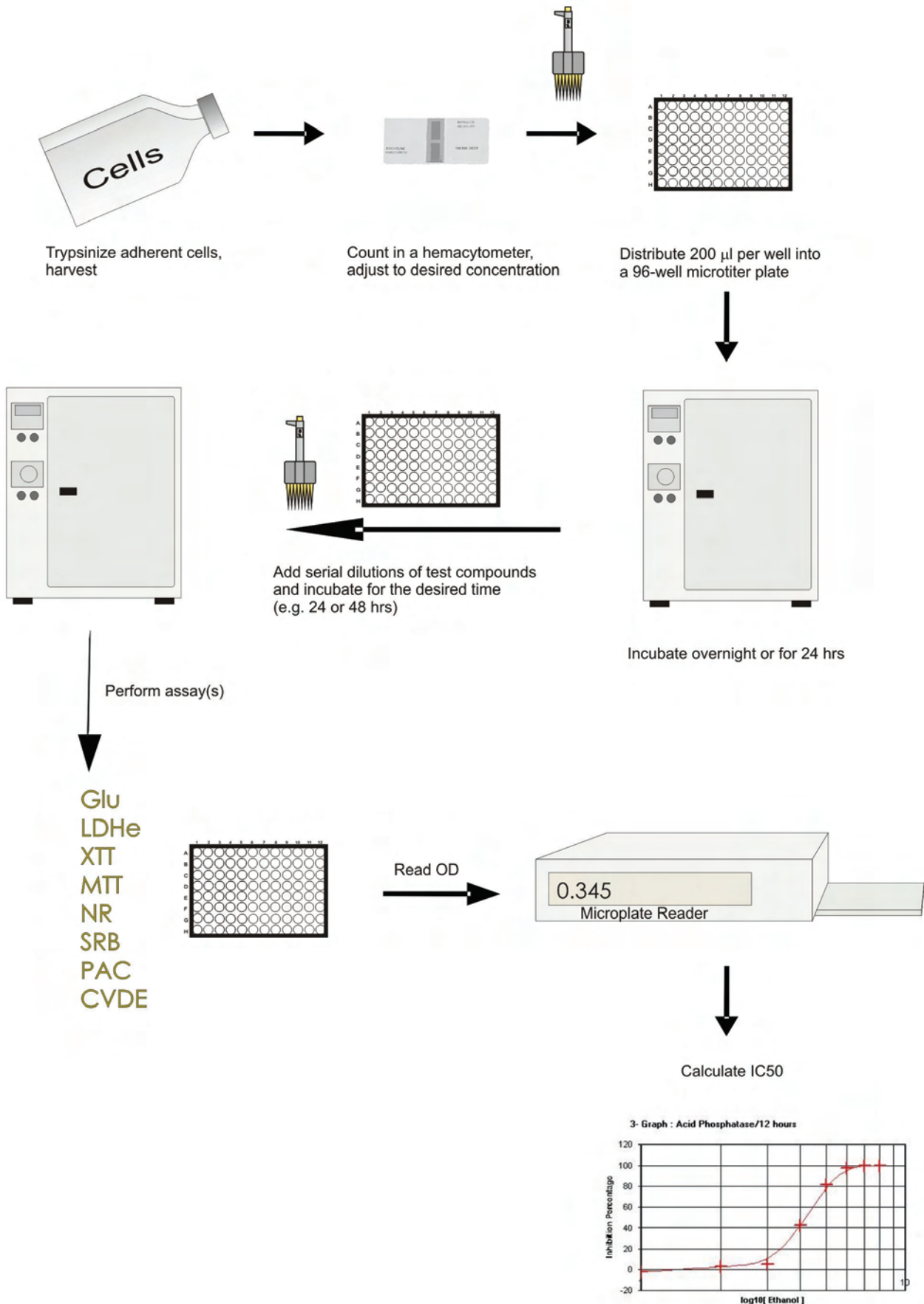
#### Multiple Parameter Kits

XTT - CVDE  
XTT - PAC  
XTT - SRB  
NR - CVDE  
NR - SRB  
SRB - CVDE  
LDHe - XTT  
LDHe - XTT - NR  
LDHe - XTT - SRB  
XTT - SRB - CVDE  
XTT - NR - SRB  
LDHe - GLU - XTT - PAC  
LDHe - GLU - XTT - SRB  
LDHe - XTT - NR - SRB

## 5. Advantages of the IN CYTOTOX Test Kits

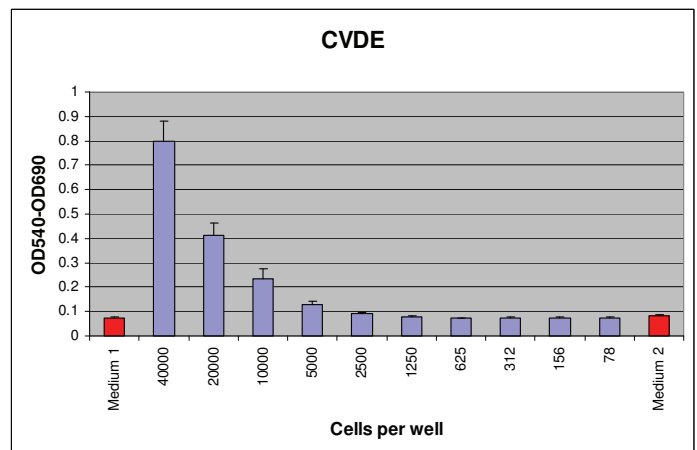
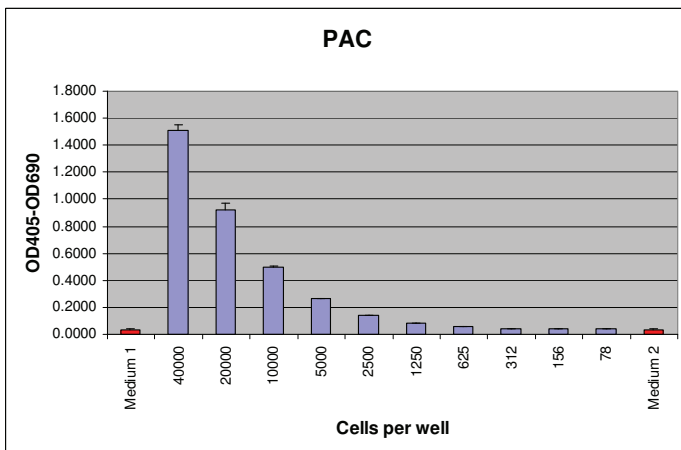
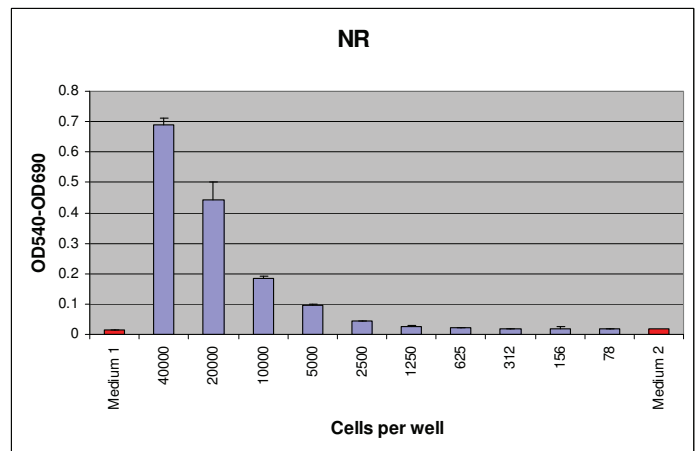
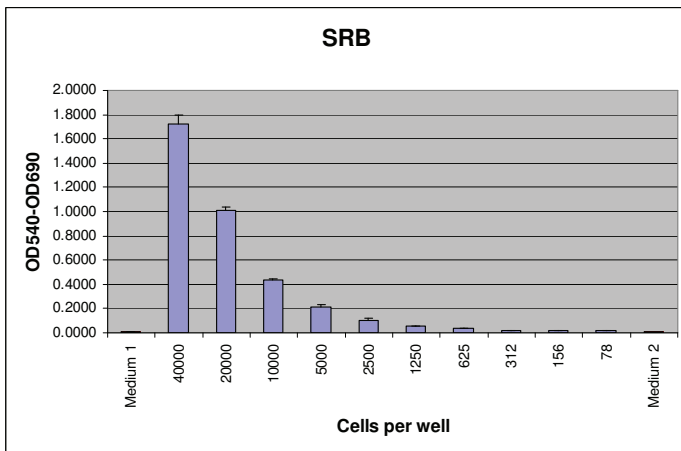
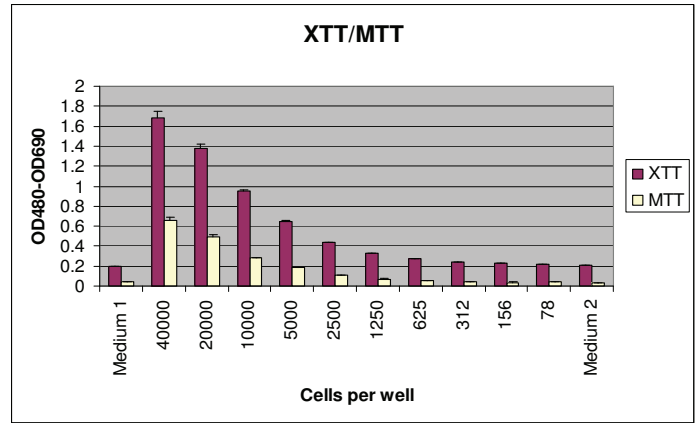
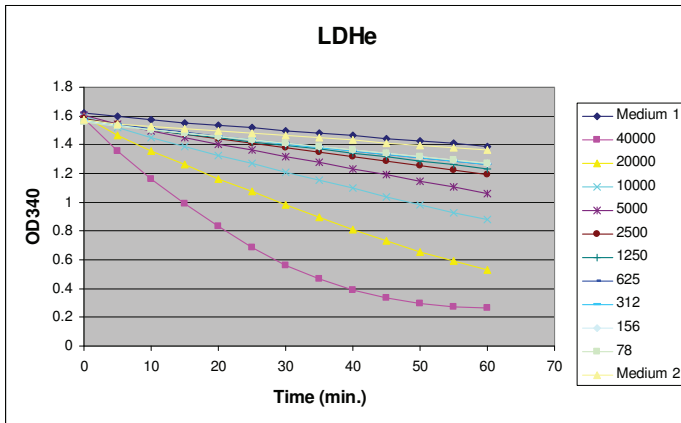
- Complete range of cytotoxicity markers
- High throughput screening methods
- Easy handling
- Single or multiple parameters from one cellular sample
- Reduced amount of test chemicals with the multiple parameter test kits
- Specific CelTox software available
- Customized reporting format
- Swiss manufacture
- Dedicated customer support by experienced staff

# In Cytotox Work Scheme



Form AI40  
08-2009

## 6. Sensitivities of In Cytotox Assays



**Sensitivity:** smallest number of cells per microtiter well giving a significant ( $p < 0.05$ ) signal above medium control.

Culture conditions: L929 mouse fibroblasts seeded in triplicates at the cell numbers indicated and grown overnight. Actual sensitivity may vary with cell type and culture conditions.

LDHe: 1250  
 XTT: 78  
 MTT: 1250  
 SRB: 156  
 NR: 625  
 PAC: 625  
 CVDE: 2500