

MTT Cytotoxicity test with plateable human hepatocytes

1. Objective

The *in vitro* Cytotoxicity test with human hepatocytes is used to identify the cytotoxic potential of a test substance.

2. Introduction

The general principle for the detection of cell growth or cell kill via the MTT cytotoxicity assay is the conversion of the tetrazolium salt (MTT) to the coloured product formazan. The concentration can be measured photometrically at 570 nm. The formation of formazan takes place via intact mitochondria. An advantage of using cultured hepatocytes is to investigate fundamental aspects of drug-metabolism-linked toxicity.

3. Method

Each well was overgrown with approximately 10^5 cells in Hepatocyte Incubation Medium (HIM) and pre-incubated for 2 days. Every 24 h the medium had to be changed.

After removal of the culture medium each well was exposed to 100 μ l HIM containing the test item or the positive/negative control, respectively, for 24 h.

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After removal of the incubation medium 100 µl of HIM^{Komplett} containing 1 mg/ml MTT will be added to each well followed by 1 - 3 hours of incubation. Afterwards the remaining dye was extracted with 100 µl/well of lysis buffer.

The optical density of the resulting supernatant was measured photometrically at 570 nm.

4. Results

The respective EC₅₀-values for the test items will be determined from the percentage of the test item relative to the negative control.

The EC₅₀ values will be calculated via the “four parameter logistic curve”:

$$f = \frac{\min + ((\max - \min))}{1 + \left(\frac{x}{EC_{50}}\right)^{Hillslope}}$$

In cases, where the “four parameter logistic curve” is not representative, the “linear regression” will be used:

$$f=y+a*x$$

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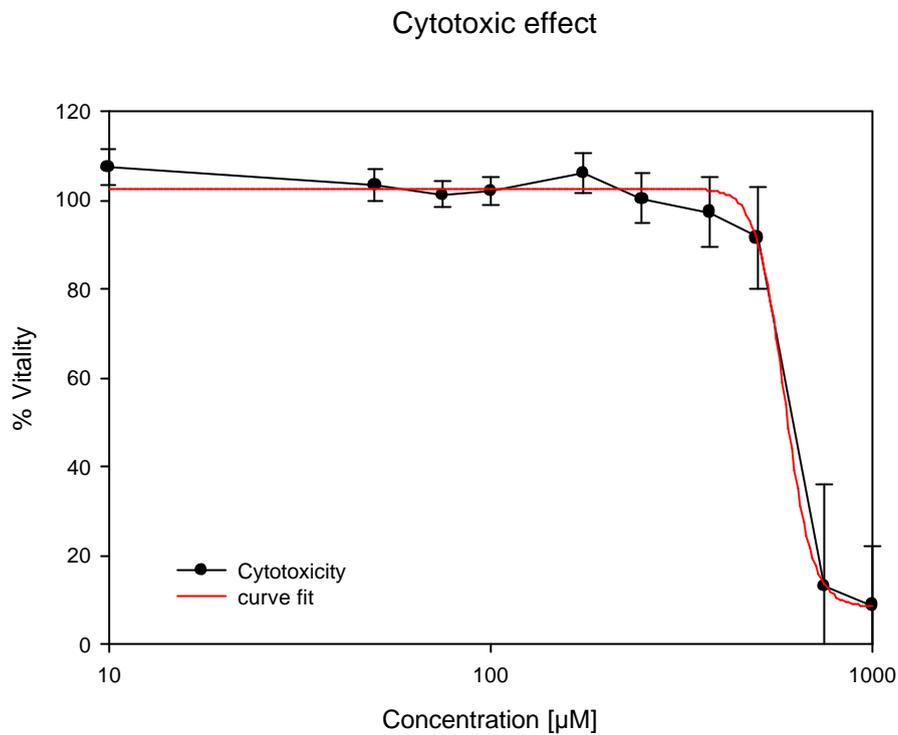


Figure 1: Example for the cytotoxic effect: $EC_{50} = 590 \mu\text{M}$

A cytotoxic potential of a test item in the tested range is assumed if

- a dose-effect relationship is observed
- a decrease of vital cells over the spread is observed

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