

## **MTT Cytotoxicity test with V79MZ Chinese hamster cells**

### **1. Objective**

The *in vitro* Cytotoxicity test with V79MZ Chinese hamster cells 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.

The V79MZ chinese hamster cells have no metabolic activity. Because of that, the cytotoxic effects of the test items on the test system will be determined in presence and in absence of an metabolic activating enzyme system.

### **3. Method**

Cells are sown out and cultured for approximately 24 h.

After removal of the culture medium each well will be exposed to the solutions of the test item, negative and positive controls. The incubation could be performed without and with metabolic activating enzyme system (rat S9 complemented with cofactors).

After the incubation time cells were stained for 1 - 3 h with MTT in complete medium.

Afterwards the remaining dye was extracted with lysis buffer.

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

For more information please contact us!

## 4. Results

The respective EC<sub>50</sub>-values for the test items will be determined from the percentage of the test item relative to the negative control.

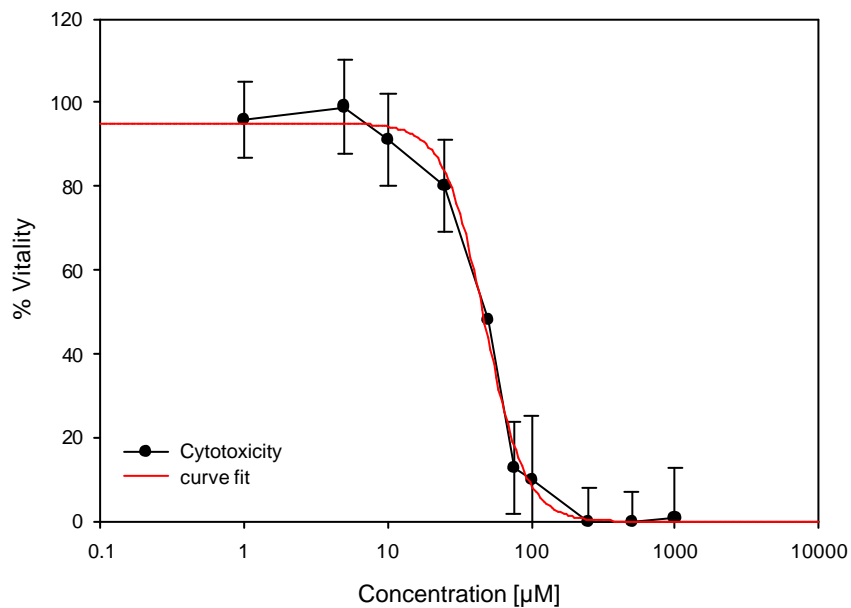
The EC<sub>50</sub> values will be calculated via the “four parameter logistic curve”:

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

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

$$f=y+a*x$$

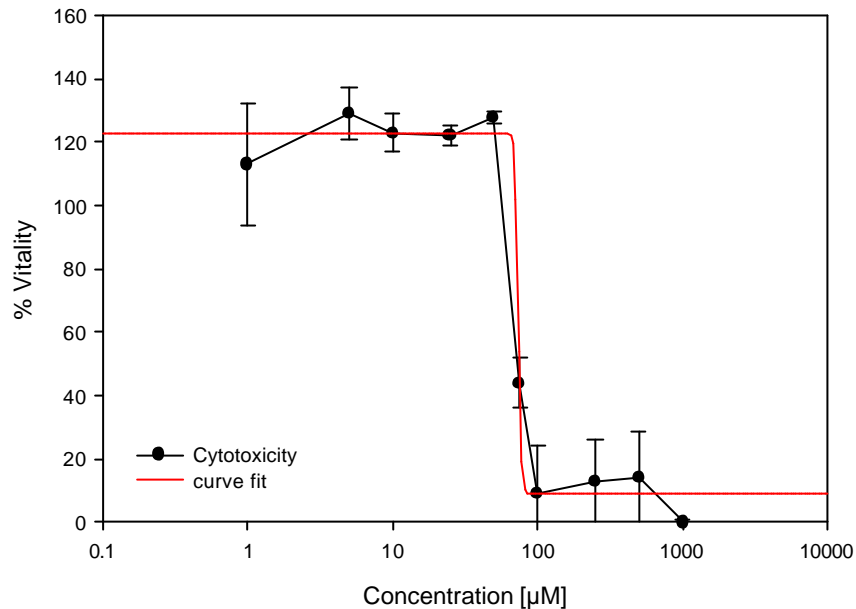
Cytotoxic effects for incubation without S9



**Figure 1: Example for the cytotoxic effect for incubation without S9: EC<sub>50</sub> = 42.0 µM**

For more information please contact us!

### Cytotoxic effect for incubation with S9



**Figure 2: Example for the cytotoxic effect for incubation with S9: EC50 = 73.6 µM**

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

For more information please contact us!