

# Measuring Cell Viability with the Thermo Scientific Multiskan GO Microplate Spectrophotometer and SkanIt software

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## Goal

This application note describes how three different cell viability assays—neutral red uptake, MTT and WST-1—were carried out on mouse fibroblast cell line Balb/c 3T3 by using the Thermo Scientific™ Multiskan™ GO microplate spectrophotometer and the Thermo Scientific™ SkanIt™ software for assay readouts and data analyses.

## Introduction

Cell viability and cytotoxicity assays *in vitro* are widely used in several disciplines to evaluate the effects of test agents on cells. Depending on the chosen cell line and assay type, these assays can be utilized for many purposes, i.e. for primary assessment of chemical toxicity or for screening of antiproliferative effects. Very commonly used assays are based on measuring the metabolic activity of cells by using tetrazolium salts that are reduced to colored formazan products by viable cells. MTT was the first tetrazolium salt used for this purpose (Mosmann, 1983), but the formazan crystals formed from MTT need to be solubilized with organic solvents before quantification, and thus several other tetrazolium salts with improved features have been developed. One of these is WST-1, which is cleaved to water-soluble formazan and thus does not require solubilization. Cell viability can also be assessed by measuring cellular uptake, for example, by using neutral red dye, which penetrates the cell membrane and accumulates into lysosomes of viable cells (Repetto et al., 2008). Neutral red uptake assay on Balb/c 3T3 cells is also a recommended *in vitro* assay for acute oral toxicity testing of chemicals according to the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM, 2013).

## Materials and Methods

### Cell Culture

Mouse fibroblast cell line, Balb/c 3T3 (ECACC 86110401), was obtained from ECACC (UK). Cells were grown in DMEM (Sigma-Aldrich, USA), supplemented with Gibco® 5% foetal bovine serum (FBS, Thermo Fisher Scientific), Gibco® 5% newborn calf serum (NCS, Thermo Fisher Scientific), 2 mM L-glutamine, 100 IU/ml penicillin and 100



µg/ml streptomycin and incubated at 37°C, 5% CO<sub>2</sub>, and 95% humidity. For the assays, cells were seeded into clear Thermo Scientific™ Nunc™ 96-well microplates (Thermo Fisher Scientific) at a density of 10,000 cells/well. After overnight incubation, cells were exposed for 24 hours to a series of polymyxin B concentrations diluted into assay media with Gibco® 5% serum.

### Neutral Red Uptake Assay

The neutral red uptake assays was carried out according to Repetto et al. (2008). Briefly, neutral red (Sigma-Aldrich, USA) stock solution (4 mg/ml in PBS) was diluted with assay media to 40 µg/ml working solution, incubated overnight at 37°C and centrifuged. Media was removed from the assay plate and 100 µl of neutral red solution was added to each well. After two hours of incubation at 37°C, the neutral red solution was removed and the cells were washed with PBS. Next, 150 µl of neutral red destain solution (50% ethanol, 49% deionized water and 1% glacial acetic acid) was added per well, the plate was shaken for 10 minutes and the absorbance at 540 nm was measured.

## MTT Assay

MTT assay was performed as previously described by Mosmann (1983). 10 µl of MTT solution (Sigma-Aldrich, USA; 5 mg/ml in sterile PBS) was added to each well, and the plate was incubated for three hours. After removing the media, the formazan crystals were dissolved with DMSO, and the absorbance was measured at 550 nm and 655 nm (background).

## WST-1 Assay

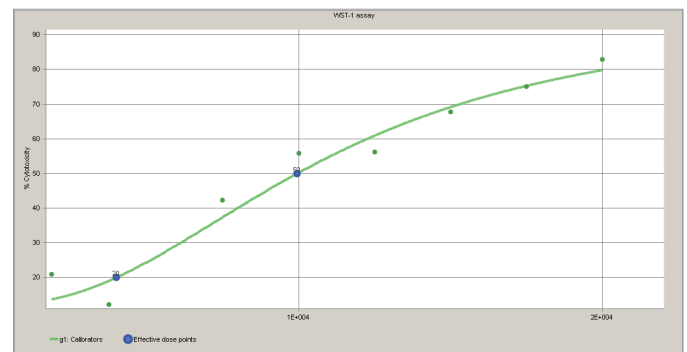
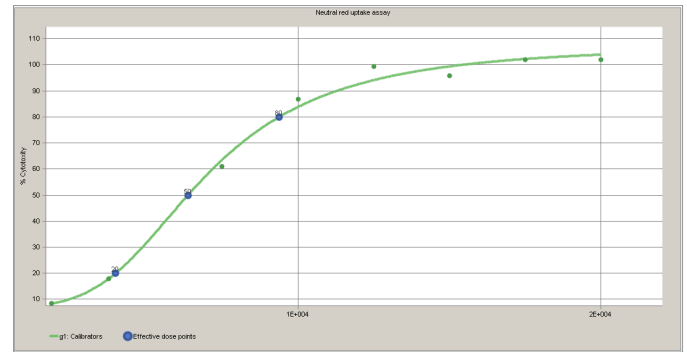
Tetrazolium salt WST-1 was obtained as premixed, ready-to-use solution from Clontech (630118, USA) and used according to manufacturer's instructions. After sample exposure, 10 µl of WST-1 solution was added to each well and the plate was incubated at 37°C for two hours. The plate was shaken for 1 minute and the absorbance was measured at 440 nm and 690 nm (background).

## Data Analysis

Data analyses were carried out using the functions available in the SkanIt software, which is provided with the Multiskan GO plate reader. The analyses included several steps, such as background and/or blank subtraction, basic statistics, normalization and EC50 calculations.

## Results and Discussion

Cell viability assays on mouse fibroblast cell line, Balb/c 3T3, were carried out using three different cell viability assays, MTT, WST-1 and neutral red uptake. Polymyxin B, a polypeptide antibiotic causing cell membrane disruption, was used as an example compound in the experiments, and displayed concentration-dependent activity in all the assays. Calculated EC50 values from the MTT, WST-1 and neutral red uptake assays for polymyxin B were in the range of 7,000–10,000 IU/ml. The similarity of the values demonstrates that these assays yield comparable data, and thus the assay selection can be done case-by-case, depending on the purpose and resources. For example, the WST-1 assay is by far the simplest to execute and thus saves the most time, but the MTT and neutral red uptake assays are more cost-effective in other terms. By using Multiskan GO for the assay readout and SkanIt software for the data analysis, processing of the results and reporting of EC50 values was greatly simplified. Figure 1 shows dose-response curves for polymyxin B from WST-1 and neutral red uptake assays fitted into four parametric logistic function for EC50 calculation.



**Figure 1:** Dose-response results for polymyxin B against Balb/c 3T3 fibroblasts after 24-hour exposure measured by **A.)** neutral red uptake and **B.)** WST-1 assay.

## References

EURL ECVAM Recommendation, 2013, available from:

<https://eurl-ecvam.jrc.ec.europa.eu/eurl-ecvam-recommendations/3t3-nru-recommendation>

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*, 1983;65: 55–63.

Repetto G, del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. *Nature Protocols*, 2008;3(7):1125-31.

[www.thermoscientific.com/platereaders](http://www.thermoscientific.com/platereaders)

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