XTT Colorimetric Assay Kit

(Cell Viability & Proliferation)

(1000 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44117-1000

www.zellx.de

Application:

Detection of Cell Viability, Proliferation & Function

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

Background

The XTT assay is an optimized colorimetric assay for assessing cell viability and proliferation in mammalian cells based on their cellular redox potential. Dehydrogenase enzymes in metabolically active cells reduce the yellow tetrazolium salt XTT to the brightly orange formazan dye which can be measured at 450 nm. Since this reduction can only occur in metabolically active cells, it is considered as an indicator of cell viability. The amount of produced formazan correlates with the number of viable cells in the sample, and an electron coupling reagent is used to improve the efficiency of XTT reduction in cells.

Intended use

Determining cell viability and proliferation in mammalian cells.

Materials supplied in the Kit

Component	*Quantity*
XTT Reagent	50 mL
Electron Coupling Reagent	1 mL

Storage instruction

All reagents should be stored at -20° C, protected from light, until the expiration date of the kit.

Upon receipt, aliquot the Electron Coupling Reagent into 10 tubes containing 100 µL and keep at -20° to avoid freezing/thawing cycles.

Materials required but not supplied

Precision pipettes and disposable filter pipette tips

Sterile clear 96 well plate

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Reagent preparation

i. **XTT Working solution:** add 100 µl of the Electron Coupling Reagent to 5 mL of XTT reagent (this amount is sufficient for one 96-well plate or 100 tests). XTT Working solution must be used immediately after preparation. Keeping at RT for extended periods of time sharply reduces the sensitivity of the assay.

Sample preparation

i. One to two days before the experiment, cultivate cells (10^4-10^5 cells/well) into a 96-well plate containing 100 µL/well of cell culture medium. Each plate must be included at least three wells as Blank which contain only complete culture medium without the cells. Cultivate the cells in a CO_2 incubator at 37°C for 24-48 hours.

➢ We strongly recommend to determine the optimal cell number and incubation time for your specific cells before performing a large number of XTT assays.

Assay Procedure

1. Add 50 µL of the XTT Working solution to each well. Mix gently for one minute.
2. Incubate the cells for 2 hours (adherent cells) to 4 hours (suspension cells) at 37°C in a CO_2 incubator.
3. Shake the plate gently to evenly distribute the generated dye in the wells.
4. Read the absorbance signal at 450 nm (450-500 nm is acceptable). Read background absorbance at 690 nm (630-690 nm is acceptable).

Calculations

i. Subtract the background absorbance from the signal absorbance to obtain normalized absorbance values.

$$\mathbf{A = A_{450\,nm}(Test) - A_{450\,nm}(Blank) - A_{690nm}(Test)}$$