TACS™ XTT

Cell Proliferation/Viability Assay

Catalog Number 4891-025-K

~2500 Tests (25 96-well microplates)

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Measurement of cell viability and proliferation comprise the underlying basis for numerous *in vitro* assays directed towards the quantitation of a cell population's response to external factors. Cell proliferation assays have utilized the uptake of radiolabeled thymidine into cellular DNA; however, this method is time consuming and involves the use of radioactive materials. The use of tetrazolium salts, including XTT (2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carbox-anilide), to assay cell proliferation, cell viability, and/or cytotoxicity is a wide-spread, established practice. The XTT assay procedure avoids radioactivity, allows for rapid determination in microplates, and gives reproducible and sensitive results.

Cleavage of the tetrazolium salt to formazan occurs via the succinate-tetrazolium reductase system in the mitochondria of metabolically active cells. The reaction is attributed mainly to mitochondrial enzymes and electron carriers, but a number of other non-mitochondrial enzymes have been implicated.

XTT, a yellow tetrazolium salt, is cleaved to a soluble orange formazan dye, which can be measured by absorbance at 490 nm (or 450 nm) in a microplate reader. Efficient reduction of XTT requires an electron coupling reagent. This kit includes both XTT and the electron coupling reagent for a convenient and simple assay.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.

PRECAUTIONS

The physical, chemical, and toxicological properties of the products in this kit have not yet been fully investigated. The use of gloves, lab coats, and eye protection while using any of these chemical reagents is highly recommended. R&D Systems assumes no liability for damage resulting from handling or contact with these products. Material Safety Data Sheets are available upon request.

XTT (CAS # 111072-31-2) is toxic and may cause heritable genetic defects. In case of contact, immediately flush eyes or skin with copious amounts of water. If swallowed, wash out mouth with water provided person is conscious. Call a physician.

PMS (phenazine methosulfate, CAS # 299-11-6) is toxic and may be carcinogenic and/or mutagenic. PMS is an irritant. In case of contact, immediately flush eyes or skin with copious amounts of water. If swallowed, wash out mouth with water provided person is conscious. Call a physician.

REAGENTS PROVIDED

XTT Reagent (Part 4891-025-01) - 5 vials (25.0 mL per vial) containing a 0.9 mg/mL solution of XTT. Store at ≤ -20° C in a manual defrost freezer. Avoid repeated freeze-thaw cycles.

XTT Activator (Part 4891-025-02) - 5 vials (0.5 mL per vial) containing PMS. Store at ≤ -20° C in a manual defrost freezer. Avoid repeated freeze-thaw cycles.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader with filters to read at 490 nm (or 450 nm) and 630 nm or greater
- Tissue culture microplates
- Cell culture media (without phenol red)
- Adjustable pipettes and pipette tips
- Trypan Blue
- PBS

REAGENT PREPARATION

XTT Reagent - Stock is provided at a 3X concentration (0.9 mg/mL) in RPMI media without phenol red. A volume of 5 mL is sufficient to run one microplate. XTT will precipitate during storage. Therefore, the solution must be warmed to 37° C for several minutes until it is no longer opaque. Heating for unnecessary and extended periods of time will result in reduction of the XTT.

XTT Activator - Stock is provided at a 50X concentration in dH_2O. A volume of 100 µL is sufficient for each 5 mL of XTT Reagent (*i.e.* one microplate). The XTT Activator will also precipitate during storage. Heat to 37° C for 2 to 5 minutes until the reagent is fully dissolved.

XTT Working Solution - Immediately before use, add 100 µL of XTT Activator to 5 mL of XTT Reagent to make the XTT Working Solution. XTT Working Solution should be added to cells within several minutes of preparation.

ASSAY PROTOCOL

1. Seed cells at varying densities (1,000 - 100,000 cells per mL) in 100 µL of an appropriate medium in microplates. Treat according to experimental protocol (varying amounts of proliferative or toxic compounds, etc.). For seeding, remember to account for length of treatment, growth rate of cells, etc. The XTT Reagent is very sensitive and if cells are overgrown, the dynamic range of the assay may be exceeded. At the same time, some cells with low metabolic rates (*e.g.* primary cells) may require a higher seeding density and/or a longer treatment procedure.
2. Prepare XTT Working Solution by combining XTT Reagent with XTT Activator according to instructions (see Reagent Preparation).
3. Add 50 µL of XTT Working Solution to each well. Appropriate incubation times with XTT Working Solution are determined empirically.
4. Read absorbance at 490 nm (or 450 nm), with a reference wavelength of 630 - 690 nm (to correct for fingerprints, smudges, etc.).

RESULTS

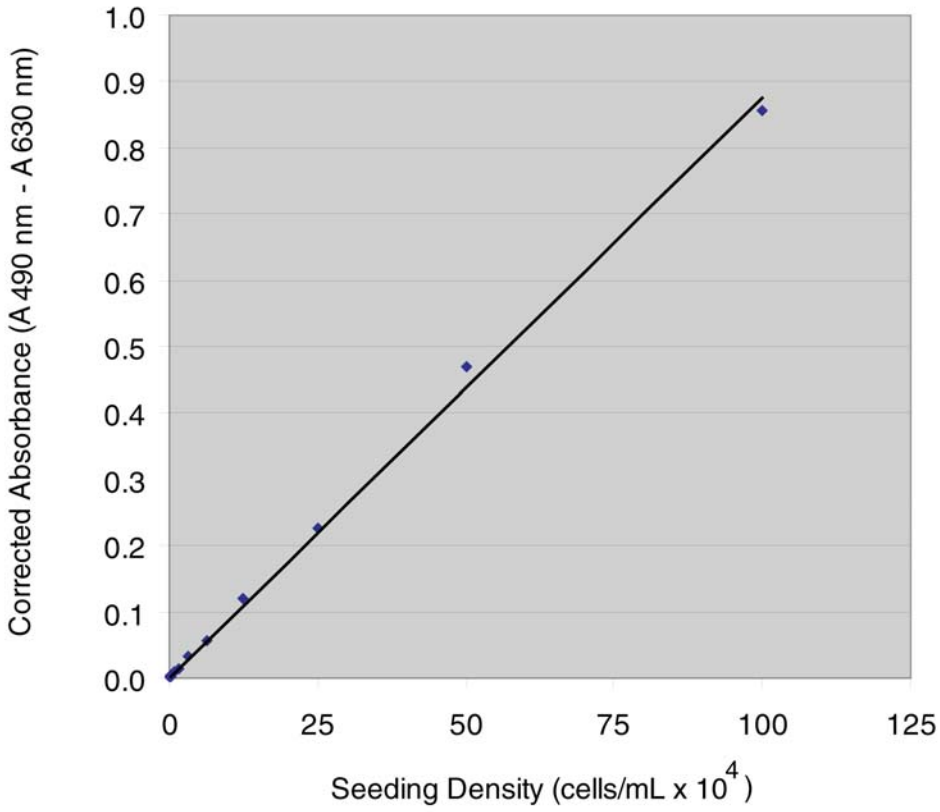

Figure 1: Wehi cells were grown in DMEM supplemented with 10% fetal bovine serum, washed with 1X PBS, and counted using Trypan blue and a hemacytometer. Cells were resuspended in RPMI (without phenol red), serially diluted in a microplate using 100 µL of the listed densities, and then incubated with the XTT Working Solution for 6 hours at 37° C under 5% CO_2. Absorbance values were obtained at 490 nm with a reference correction at 630 nm in an ELISA plate reader.

STANDARDIZATION

There are two measurement options: (1) measure relative differences or (2) compare absolute cell number.

To monitor relative changes in cell number in the same cell type, it is not necessary to calibrate the system. Data may be presented as the percent change in absorbance relative to an experimental control.

To calibrate using cell number, determine the cell number in a sample and plate out dilutions in triplicate covering a range of 1,000 - 100,000 cells per mL in 100 μL of medium. Perform the standard assay. Determine averages of triplicate values and plot data as cell number per well versus absorbance.

TROUBLESHOOTING GUIDE

Problem	Action
Low absorbance readings	Prepare the XTT Working Solution immediately before use. Increase incubation time with the XTT Working Solution. Increase seeding density of cells. Ensure the XTT Reagent and Activator are in solution before beginning the assay.
Poor replicates	Ensure no bubbles are present in wells. Pipette cells and/or XTT Working Solution accurately. Check the accuracy of the pipette. Ensure XTT Reagent and/or XTT Activator are fully dissolved before use.
High background	Check proper storage of XTT at ≤ -20° C in a manual defrost freezer. Use freshly made XTT Working Solution. Decrease incubation time with XTT. Ensure media is free of microbial contamination. Serum will contribute to reduction of XTT. If possible, eliminate or reduce serum before adding XTT Working Solution.

REFERENCES

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2. Stevens, M.G. and S.C. Olsen (1993) J. Immunol. Methods **157**:225.
3. Scudiero, D. *et al.* (1988) Cancer Res. **48**:48274.
4. Puissant, A. *et al.* (2008) FASEB J. **22**:1894.

NOTES