TREVIGEN® **Instructions**

For Research Use Only. Not For Use In Diagnostic Procedures

TACS® XTT
Cell Proliferation Assay

Cat# 4891-025-K, 2500 Tests

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I. Background

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 hazardous 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 (or 450) nm in a microplate reader. Efficient reduction of XTT requires an electron coupling reagent. Trevigen's **TACS® XTT kit** includes XTT and an electron coupling reagent for a more rapid, convenient and simple assay.

Advantages/Features:

- Sensitive
- No radioactivity
- Rapid (no solubilization step as in an MTT assay)
- Ideal for high throughput assays (no washing or other steps that can cause cell loss and variability)

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.

2. The physical, chemical and toxicological properties of the provided products may not yet have been fully investigated; therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products. MSDS sheets are available upon request.

3. XTT Reagent (Cat# 4891-25-01) contains a 0.9 mg/ml solution of XTT (2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, CAS # 111072-31-2). XTT 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.

4. XTT Activator (Cat. # 4891-25-02) contains PMS (phenazine methosulfate, CAS # 299-11-6). PMS 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.

III. Materials Supplied

Cat# 4890-25-K

Component	Amount Provided	Storage	Catalog #
XTT Reagent	5 x 25 ml	-20 °C	4891-025-01
XTT Activator	5 x 0.5 ml	-20 °C	4891-025-02

IV. Materials/Equipment Required But Not Supplied

Equipment

1. Microplate (ELISA) reader: 490 (or 450) and 630-690 nm filters
2. inverted microscope
3. multichannel pipette
4. pipette aid
5. 37 °C incubator
6. laminar flow hood

Reagents

1. cell culture medium (best without phenol red)
2. tissue culture microplates
3. sterile tubes (5 ml)
4. serological pipettes
5. sterile pipette tips

V. Reagent Preparation

XTT Working Solution is created by mixing two reagents: XTT Reagent and the XTT Activator; follow step 3 below:

1. **XTT Reagent**
   The stock is provided at a 3X concentration (0.9 mg/mL) in RPMI medium 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.

2. **XTT Activator**
   The stock is 50X in dH_2O. A volume of 100 µL is sufficient for each 5 mL of XTT reagent (i.e. one microplate). Like the XTT reagent, the XTT activator will precipitate during storage. Heat to 37 °C for 2 to 5 minutes until the reagent is fully dissolved.

3. **XTT Working Solution**
   Immediately before use, add 100 µL of the XTT activator to 5 mL of XTT reagent to make XTT Working Solution. Prepared XTT Working Solution should be added to cells within several minutes.

VI. Assay Protocol

1. Seed cells at varying densities (1,000-100,000 cells per mL) in 100 µL of 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 longer treatment procedure.

2. Prepare XTT Working Solution by combining XTT Reagent with XTT Activator according to instructions (See Section IV, Reagent Preparation, Step 3, above)

3. Add 50 µL of prepared XTT Working Solution to each well.

4. Read absorbance at 490 nm (or 450 nm), with a reference wavelength of 630-690 nm (to correct for fingerprints, smudges, etc.).

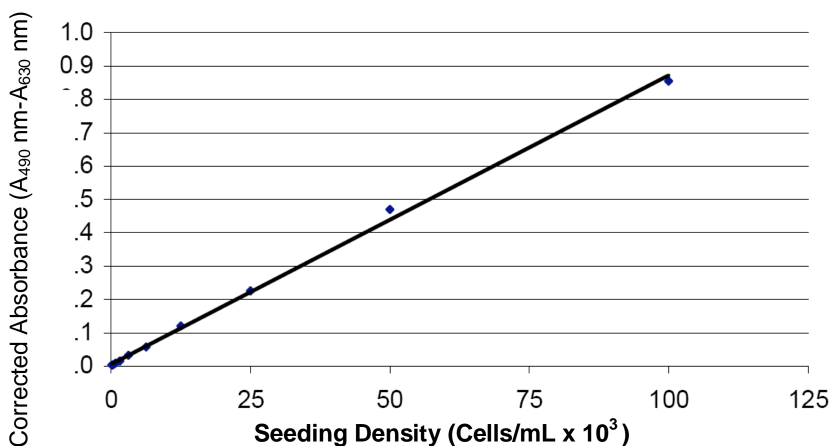

Figure 1. Quantitation of Wehi cells with XTT

Wehi cells were grown in DMEM supplemented with 10% FBS, washed with 1X PBS, and counted using Trypan blue and a hemacytometer. Cells were resus-pended 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.

VII. Standardization

There are two options for the reference system: measure relative differences or 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 to 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.

VIII. Data Interpretation and Troubleshooting

Problem	Solution
Low absorbance readings	♦ Add activator immediately before use ♦ Increase incubation time with XTT ♦ Increase seeding density of cells ♦ Ensure XTT is in solution before beginning assay
Poor replicates	♦ Ensure no bubbles present in wells ♦ Pipet cells and/or XTT solution accurately ♦ Check accuracy of pipettor ♦ Ensure XTT Reagent and/or XTT Activator are fully dissolved before use
High Background	♦ Check proper storage of XTT at –20 °C ♦ Use freshly made XTT 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

IX. References

1. Roehm NW, Rodgers GH, Hatfield SM, Glasebrook AL. (1991) An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT. *J Immunol Methods* **142**:257-65.
2. Stevens MG, Olsen SC. (1993) Comparative analysis of using MTT and XTT in colorimetric assays for quantitating bovine neutrophil bactericidal activity. *J Immunol Methods* **157**:225-31.
3. Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, Currens MJ, Seniff D, Boyd MR. (1988) Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res* **48**:48274-33.
4. Puissant A, Grosso S, Jacquel A, Belhacene N, Colosetti P, Cassuto J-P, Patrick A. (2008) Imatinib mesylate-resistant human chronic myelogenous leukemia cell lines exhibit high sensitivity to the phytoalexin resveratrol. *FASEB J* **22**:1894-904.

X. Related Products

Catalog #	Description	Size
4890-025-K	TACS® MTT Cell Proliferation Assay	2500 tests
3447-096-K	Cultrex® 3D Culture 96 Well Collagen I Cell Proliferation Assay	96 tests
3446-096-K	Cultrex® 3D Culture Laminin I Cell Proliferation Assay Kit	96 tests
3445-096-K	Cultrex® 3D Culture BME Cell Proliferation Assay Kit	96 tests
4892-010-K	Cultrex® Calcein-AM Cell Viability Kit	1000 tests
4817-60-K	FlowTACS™ Apoptosis Detection Kit	60 samples
4822-96-K	HT TiterTACS™ Assay Kit	96 tests
4830-01-K	TACS® Annexin V FITC Kit	100 samples
4835-01-K	TACS® Annexin V Biotin Kit	100 samples
6300-100-K	DePsipher™ Mitochondrial Potential Assay Kit	100 tests
6305-100-K	MitoShift™ Mitochondrial Potential Assay Kit	100 tests

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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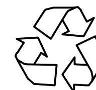

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