XTT Cell Viability Assay Kit (Colorimetric)

08/21

(Catalog # K2108-100,-1000,-2500; 100, 1000, or 2500 assays; Store at -20 °C)

I. Introduction:

BioVision's XTT Cell Viability Assay Kit is a colorimetric assay for studying cell proliferation and cell cytotoxicity. The assay is based on the reduction of the XTT tetrazolium salt by NADH, which is produced by the mitochondrial dehydrogenase enzymes of metabolically active cells to generate an orange-colored formazan product in the cell media. This kit can be used for studying cell proliferation in response to cytokines, nutrients, growth factors and mitogens and also for analyzing the cytotoxic and cytostatic effects of drugs, toxins, antibodies and other exogenous chemicals. In this assay, the XTT working solution is added directly to the cell culture media and is read after an incubation of 0.5-4 hr. The measured absorbance at 475 nm is proportional to the number of viable cells. There are no steps in the assay involving changing of media or washing or addition of any solubilization reagents. The assay can detect cell numbers as high as 500,000 viable cells and as low as 1000 viable cells in each well. The presence of phenol red and/or serum in the cell growth media does not interfere with the assay. The kit provides a safe, easy-to-use, non-radioactive, high-throughput method for characterizing and screening cell viability and cytotoxicity**.**

II. Applications:

- Measurement of cell viability and growth in response to growth factors, cytokines, mitogens and nutrients.
- Analysis of cytotoxic and cytostatic effects of drugs and toxins, antibodies and other exogenous chemicals.
- Assessment of physiological mediators and antibodies that inhibit cell growth.

III. Sample Types:

- Proliferating and non-proliferating cells
- Adherent and suspension cells

IV. Kit Contents:

Kit Components	K2108-100	K2108-1000	K2108-2500	Cap Code	Part Number
XTT Reagent Electrocoupling Solution	5 ml 100 µl	50 ml 1 ml	50 ml x 3 bottles 1 ml x 2 vials	NM/Amber Green	K2108-XX-1 K2108-XX-2

V. User Supplied Reagents and Equipment:

- Cells and cell culture media
- Cell culture hood and temperature-controlled CO_2 incubator
- 96 well clear bottom plate (sterile, cell culture grade)
- Microplate reader capable of measuring absorbance at 475 nm

VI. Reagent Preparation and Storage Conditions:

Store the kit at -20 °C, protected from light. Centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

- **XTT Reagent:** Store at -20 °C, protected from light. Avoid repeated freeze-thaw cycles. Warm to 37 °C and mix well prior to use.
- **Electrocoupling Solution:** Store at -20 °C, protected from light. Avoid repeated freeze/thaw cycles. Warm to 37 °C and mix well prior to use.
- **Preparation of the XTT Reagent working solution:** For one 96 well plate**,** add 0.1 ml of the pre-warmed Electrocoupling Solution to 5 ml of the pre-warmed XTT reagent. Mix thoroughly. **Notes:** Prepare fresh prior to use. Solution should be clear and not cloudy. If cloudy, warm to 37 °C and mix again.

VII. Cell Proliferation and Viability Assay Protocol:

1. Assay Protocol:

a. Seed the optimized number of cells in a sterile 96-well, clear bottom tissue-culture microplate. Incubate cells with the desired dose(s) of the test compounds or without any treatment or solvent controls for the desired period of time (usually 20 to 48 hr) at 37 °C in standard cell culture conditions. Include a **Background Control** well containing only the complete growth media for blank absorbance readings. The final volume of the culture medium in each well should be **100 µl.**

b. Prepare the XTT Reagent working solution. Add 50 µl of the XTT working reagent solution to each well. Mix by gently tapping the sides of the plate.

c. Incubate the plate at 37 °C for 0.5-4 hr depending on the cell type and cell density.

d. Read the plate at 30 min intervals by measuring the absorbance at 475 nm until the signal is in the desired absorbance range. Gently tap the plate to ensure that the dye is thoroughly mixed into the solution prior to each reading.

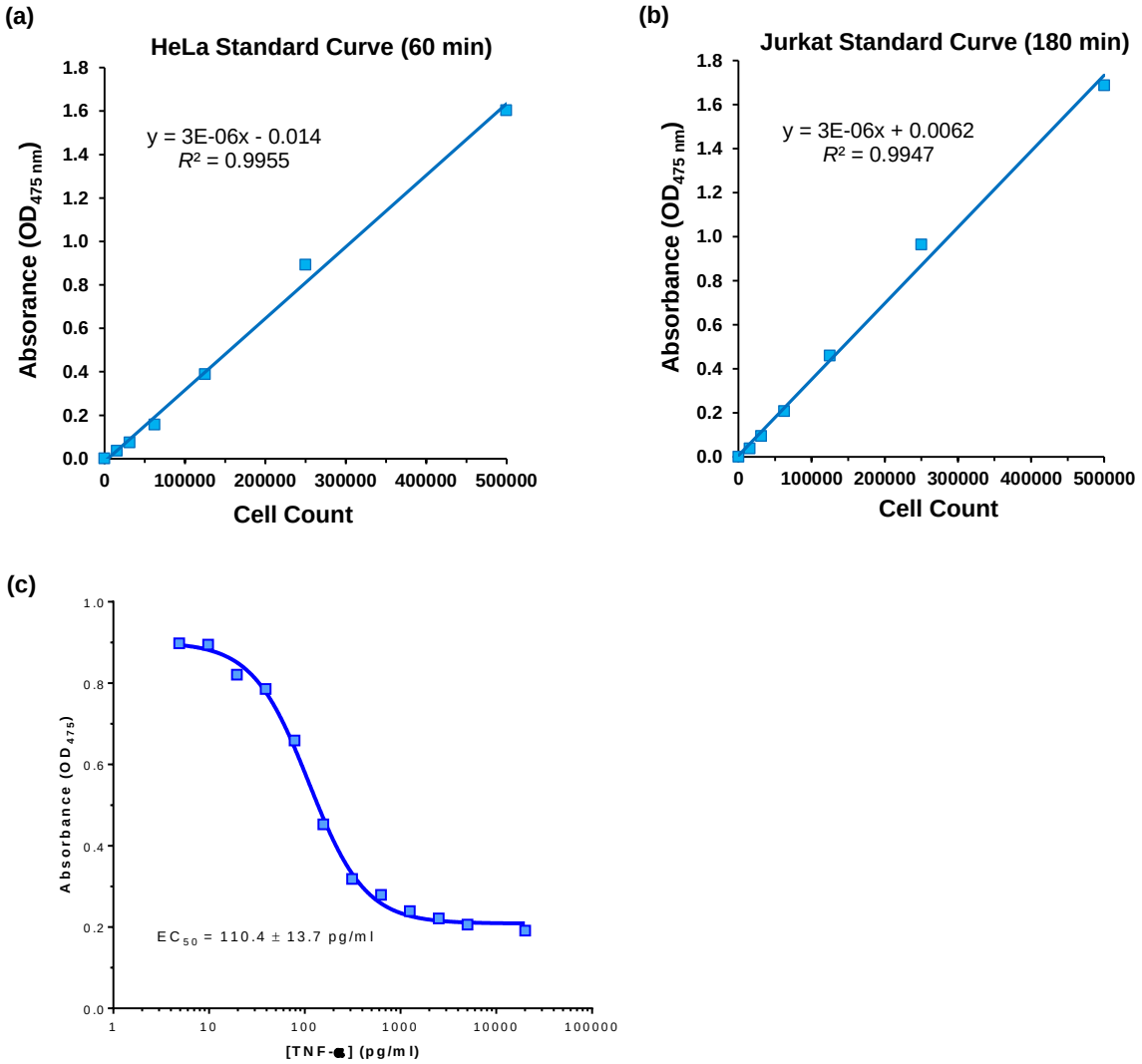
2. Calculation: Subtract the blank absorbance from all the OD 475 nm readings to obtain the normalized absorbance values

Notes:

- This assay was developed with HeLa (adherent) and Jurkat (suspension) cells and can be modified for any cell line. Growth conditions, optimal number of cells seeded per well and treatment times should be adjusted based upon the cell line used.
- Appropriate incubation time depends on the individual cell type and cell concentrations used. Therefore, it is recommended to determine the optimal cell counts (usually between 10^3 and $5x10^5$ cells) and optimal incubation time for your specific cells. Optimization

is important because the best assay results are obtained in the linear region of the curve. Cells with low metabolic activity may require higher cell concentrations and/or longer incubation times.

- For toxicity studies, it is recommended to start with more cells. Prepare parallel well(s) as **Solvent Control(s)** if the test compounds are dissolved in non-aqueous solvent (such as DMSO or acetonitrile).



Figures. (a) Standard Curve of viable HeLa cells (0 to 500,000 cells/well). **(b)** Standard Curve of viable Jurkat cells (0 to 500,000 cells/well). **(c)** Cytotoxicity dose-response curve of L929 cells (2 x 10^4 cells/well) following 48 hr treatment with TNF-α (EC_{50} = 110.4 pg/ml calculated by 4-parameter logistic curve fitting).

VIII. Related Products:

MTT Cell Proliferation Colorimetric Assay Kit (K299)
Quick Cell Proliferation Colorimetric Assay Kit (K301)
VisionBlue™ Quick Cell Viability Fluorometric Assay Kit (K303)
BrdU Cell Proliferation Assay Kit (K306)
EZViable™ Calcein AM Cell Viability Assay Kit (F) (K305)
MTS Cell Proliferation Colorimetric Assay Kit (K300)
Quick Cell Proliferation Colorimetric Assay Kit plus (K302)
Ready-to-use Cell Proliferation Reagent, WST-1 (K304)
3D Cell Culture HTS Cell Viability Complete Assay Kit (K948)

FOR RESEARCH USE ONLY! Not to be used on humans.