XTT Cell Proliferation Assay Kit

RUO *Research Use Only* REF *15091* Σ 500 -25 ℃ ~ -15 ℃

DESCRIPTION

- Cell proliferation assay is widely used in cell biology for the study of growth factors, cytokines or media components.
- XTT, which is Tetrazolium salt, is especially useful for assaying the quantification of viable cells.
- XTT Assay Kit measures cell viability based on activity of mitochondria enzymes in live cells that reduce XTT and are inactivated shortly after cell death.
- The formazan dye formed in the assay is soluble in aqueous solution and can be quantified by measuring the absorbance at wavelength 450nm - 500nm using a spectrophotometer.
- XTT Assay Kit's protocol is simple, accurate, and sensitive.

INTRODUCTION

Measurement of cell viability and proliferation comprises the underlying basis for numerous *in vitro* assays directed towards the quantitation of a cell population's response to external factors. The use of tetrazolium salts, including XTT (2,3-Bis(2- methoxy-4-nitro-5 sulfophenyl)-2Htetrazolium- 5-carbox-anilide), to assay cell proliferation, cell viability, and/or cytotoxicity is a wide-spread and 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 also have been implicated. XTT is cleaved to a soluble orange formazan dye, which can be measured by absorbance at 450 - 500nm in a microplate reader. Efficient reduction of XTT requires an electron coupling reagent. This kit includes both XTT and Activation solution for a convenient and simple assay.

KIT CONTENTS

Label	Contain
XTT Reagent	5 ml x 5 Bottle
Activation Solution	500 µl

STORAGE AND STABILITY

- Storage condition : The components of the kit are stable at -15 to -25°C, protected from light until the expiration date printed on the label. Thaw reagents immediately before use. It is recommended to prepare appropriate aliquots and to avoid repeated thawing and freezing.

  Note: Precipitates will form during shipment or storage at -15 to -25°C, in which case the container should be warmed to 37°C and mixed to obtain a clear solution.

ADDITIONAL REQUIRED EQUIPMENT

- Spectrophotometer (ELISA reader)
- Cell Culture Plate (96well)

APPLICATIONS

- Cell Viability, Proliferation & Function
- Cell Cytotoxicity

NOTICE BEFORE USE

XTT Cell Proliferation Assay Kit is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. XTT Cell Proliferation Assay Kit is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals.

PROTOCOL

1. The cells should be cultivated in a flat 96-well plate. To each well add 100 µl of growth medium. The cells should be incubated in a CO_2 incubator at 37°C. In most cases cells can be used to assay proliferation after 24 – 96 hours.
2. Defrost the XTT Reagent and the Activation Solution immediately prior to use in a 37°C bath. Swirl gently until clear solution is obtained.
3. To prepare a XTT mixture sufficient for one plate (96 wells), add 100 µl Activation Solution to 5 ml of XTT Reagent.

   Note: Depending on the number of test, mix activation solution to XTT Reagent with proportion of 50:1
4. Add 50 µl of the XTT mixture to each well and incubate the plate in an incubator for 2 – 24 hours depending on cell density and the characteristics of the cell. (usually, 2- 5 hours are sufficient).
5. Shake the plate gently to evenly distribute the dye in the wells.
6. Measure the absorbance of the samples against a background control as a blank with a spectrophotometer (ELISA reader) at a wavelength of 450 – 500 nm. In order to measure reference absorbance (to measure nonspecific readings), use a wavelength of 630 – 690 nm and subtract from the 450 – 500 nm measurement.

TROUBLE SHOOTING GUIDE

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

ORDERING INFORMATION

Product Name	Amount	Cat. No.
iN-fect™ *in vitro* Transfection Reagent	500 µl	15081
MTT Cell Proliferation Assay Kit	1000 rxn	21180
i-poly Cell Culture Plate (96well)	50 ea/cs	IPY-31096

Technical support : +82-505-550-5600

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