Cell Proliferation Kit XTT

Kit for the quantitating of cell proliferation and viability without using radioactive isotopes; 1000 assays
Product code A8088,1000

1.) Introduction

Cell proliferation assays are widely used in cell biology for the study of growth factors, cytokines and media components, for the screening of cytotoxic agents and for lymphocyte activation.
The need for a reliable, sensitive and quantitative assay that would enable analysis of a large number of samples led to the development of methods, such as:

- **use of radioactive thymidine to label DNA in living cells**
- **use of BrdU to label DNA in living cells (as a substitute for radioactive thymidine)**

The above methods have a number of disadvantages, including: use of radioactive materials and relatively complex techniques. The use of tetrazolium salts, such as MTT, commenced in the 1950s, is based on the fact that live cells reduce tetrazolium salts into colored formazan compounds. The biochemical procedure is based on the activity of mitochondrial enzymes which are inactivated shortly after cell death. This method was found to be very efficient in assessing the viability of cells. A colorimetric method based on the tetrazolium salt, XTT, was first described by P.A. Scudiero in 1988. Whilst the use of MTT produced a non-soluble formazan compound which necessitated dissolving the dye in order to measure it, the use of XTT produces a soluble dye. The use of XTT greatly simplifies the procedure of measuring proliferation, and is, therefore, an excellent solution to the quantitating of cells and their viability without using radioactive isotopes. This kit was developed to assay cell proliferation in reaction to different growth factors, cytokines and nutrient components. In addition, it is suitable for assaying cytotoxicity of materials such as TNF or other growth inhibitors. XTT can be used as a non-radioactive substitute for cytotoxic tests based on the release of ^{51}Cr from cells with no less sensitivity.

The advantages of using this kit can be summarized with the following attributes:

- *easy-to-use*: **There is no requirement for additional reagents and/or cell washing procedures.**
- *Speed*: **One step process with results within 2 – 5 hours.**
- *Sensitivity*: **Can be assayed even in low cell concentrations.**
- *Accuracy*: **Dye absorbance is proportional to the number of live cells in each well.**
- *Safety*: **There is no need for radioactive isotopes.**
- *Convenience*: **No instrumentation required except for a spectrophotometer (ELISA reader). The entire assay can be performed directly in a microtiter plate.**

2.) Kit Components

XTT Reagent (10 x 5ml)

Activation Reagent (2 x 0.5ml) containing PMS (N-methyl dibenzopyrazine methyl sulfate).

Storage: -20°C; shipment on dry ice

Stability: min. one year

Both sterile solutions should be stored frozen and should not be exposed to light. To avoid freeze-thaw cycles it is recommended to aliquot the solutions after initial thawing.

Note: If sediment is present in the solutions, warm the solutions to 37°C and swirl gently until clear solutions are obtained.

3.) Assay Principles

The assay is based on the ability of metabolically active cells to reduce the tetrazolium salt XTT to the orange colored compounds of formazan. The dye formed is water soluble and the dye intensity can be read at a given wavelength in a spectrophotometer. The intensity of the dye is proportional to the number of metabolically active cells. The use of multiwell plates and an ELISA reader enables testing a large number of samples and obtaining rapid results. The test procedure includes cultivation of cells in a 96-well plate, adding the XTT reagent and incubating for 2 - 24 hours. During the incubation time, an orange color is formed, the intensity of which can be measured with a spectrophotometer, in this instance, an ELISA reader. The greater the number of active cells in the well, the greater the activity of mitochondrial enzymes, and the higher the concentration of the dye formed, which can then be measured and quantitated.

4.) Procedure

4.1 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. Each test should include a blank containing complete medium without cells (see 4.7 background control)

4.2 Defrost the XTT reagent solution and the activation solution immediately prior to use in a 37°C bath. Swirl gently until clear solutions are obtained.

4.3 To prepare a reaction solution sufficient for one plate (96 wells), add 0.1 ml activation solution to 5 ml of XTT reagent.

4.4 Add 50 µl of the reaction solution to each well and incubate the plate in an incubator for 2 - 24 hours depending on cell density and the characteristics of the cells being analyzed. (usually, 2 - 5 hours are sufficient).

4.5 Shake the plate gently to evenly distribute the dye in the wells.

4.6 Measure the absorbance of the samples against a background control as a blank with a spectro-photometer (ELISA reader) at a wavelength of 450 - 500 nm. In order to measure reference absorbance (to measure non-specific readings), use a wavelength of 630 – 690 nm and subtract from the 450 - 500 nm measurement.

4.7 **Background control (blank)**: Slight spontaneous absorbance around 450-500 nanometer occurs in the culture medium incubated with the XTT reagent. This background absorbance depends on the culture medium, pH, incubation time and length of exposure to light. Prepare one or more blank control wells without cells by adding the same volume of culture medium and XTT Reagent solution as used in the experiment. Subtract the average absorbance of the blank control wells from that of the other wells.

5.) Notes

5.1 Defrost and prepare the reaction mixture only immediately prior to use.

5.2 Since the test is extremely sensitive, it is possible to use a low concentration of cells in the wells (approximately 5000 cells per well). Since there are cell types which show low metabolic activity, such as lymphocytes, keratinocytes and melanocytes, it is recommended to increase the concentration of cells to 2.5×10^5 cells per well, in order to obtain development of formazan color within a reasonable period of time.

5.3 Incubation time with the reaction mixture varies according to the type and concentration of the cells. Therefore, it is advisable to perform an initial test by reading the absorbance at various time lapses, i.e. after 4, 6, 8, 12 hours using the same plate.

5.4 Prior to reading the absorbance with a spectrophotometer, the plate should be gently shaken in order to evenly distribute the dye in the wells.

5.5 If the volume of the medium in each well is larger than 100 µl, add a larger amount of reaction mixture by the same increment (i.e. 100 µl reaction mixture to 200 µl growth medium).

5.6 Phenol red does not interfere with the XTT assay. Absorbance from substrate blank (medium without cells) should be substracted from the readings.

6.) Summary of Proliferation Assay Using XTT Reagent: Flow Chart

- Defrost XTT reagent and activation reagent (37°C)
- Prepare reaction mixture (0.1 ml activation reagent and 5 ml XTT reagent for one plate)
- Add 50 µl reaction mixture for each well (containing 100 µl medium)
- Incubate at 37°C for 2 - 24 hours (in most cases incubation for 2 - 5 hours is sufficient)
- Measure absorbance at a wavelength of 450 - 500 nm (reference absorbance at a wavelength of 630 - 690 nm)

7.) References

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