BI
Biological Industries

Cell Proliferation Kit (XTT based)

Cat. No.: 20-300-1000 (for 1000 Assays)
Store at: -20°C

Instructions for Use

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 live cells
- Use of BrdU to label DNA in live 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 and is based on the fact that live cells reduce tetrazolium salts into colored formazan compounds. The biochemical procedure is based on the activity of mitochondria 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[4]. 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.

Advantages:

Easy-to-use. There is no requirement for additional reagents and/or the cell washing procedures

Speed. Multiwell plates and an ELISA reader can be used for reading

Sensitivity. Can be assayed even in low cell concentrations

Accuracy. Dye absorbance is proportional to the number of cells in each well

Safety. There is no need for radioactive isotopes

Kit Components

1. XTT Reagent (10x5ml)

A sterile solution containing the XTT reagent. The solution should be stored frozen and should not be exposed to light. To avoid repeated re-freezing, dividing the solution into a number of vials after defrosting the original vial is recommended.
Note: if sediment is present in the solution, heat the solution to 37°C and swirl gently until a clear solution is obtained.

2. Activation Reagent (2x0.5ml)

A sterile solution containing PMS (N-methyl dibenzopyrazine methyl sulfate). The solution should be stored frozen and should not be exposed to light. To avoid repeated re-freezing, dividing the solution into a number of vials after defrosting the original vial is recommended.
Note: if sediment is present in the solution, heat the solution to 37°C and swirl gently until a clear solution is obtained.

Assay Principles

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

Procedure

1. The cells should be cultivated in a flat 96-well plate. To each well add 100µl of growth media. 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 7: background control).
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.
3. To prepare a reaction solution sufficient for one plate (96 wells), add 0.1ml activation solution to 5ml XTT reagent.
4. Add 50µl of the reaction solution to each well and incubate the plate in an incubator for 2-24 hours (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 (see 7) with a spectrophotometer (ELISA reader) at a wavelength of 450-500 nanometer. In order to measure reference absorbance (to measure non-specific readings), use a wavelength of 630-690 nanometer and subtract from the 450-500 nanometer measurement.
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.

Notes

1. Defrost and prepare the reaction mixture only immediately prior to use.
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 $2.5x10^5$ cells per well, in order to obtain development of formazan color within a reasonable period of time.
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.
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. If the volume of the media in each well is larger than 100µl, add a larger amount of reaction mixture by the same increment (i.e. 100µl reaction mixture to 200µl growth media).

References

1. Hansen, M.B., et al, (1989), J. Immunol. Meth. 119, 203-210
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4. Scudiero, P.A., et al, (1988), Cancer Res. 48, 4827-4833
5. Tada, H., et al, (1986), J. Immunol. Meth. 93, 157-165
6. Weislow, O.S., et al, (1989), J. Natl Cancer Inst. 81, 577-586

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