Colorimetric Cell Viability Kit III (XTT)

PromoKine

Instruction Manual

Product Name	Product Description	Size	Catalog Number
Colorimetric Cell Viability Kit III (XTT)	Colorimetric Cell Viability Kit III (XTT)	1000 assays 200 assays	PK-CA20-300-1000 PK-CA20-300-200

Introduction

Cell viability and 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. 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 for quantitating cells and detemining their viability without using radioactive isotopes. This kit was developed to assay cell proliferation as response 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 loss of 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 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 (PK-CA20-300-200: 1 x 10 ml; PK-CA20-300-1000: 5 x 10 ml)

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 PMS (PK-CA20-300-200: 1 x 50 µl; PK-CA20-300-1000: 5 x 50 µl)

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.

Storage

Store at –20°C.

Assay Principles

The assay is based on the ability of metabolic active cells to reduce the tetrazolium salt XTT to orange-colored formazan compounds. The dye formed is water-soluble and its 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 allows for 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 the intensity of which 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 100 µl of growth media (medium may contain up to 10% serum). 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. In general, cells should be seeded at densities between 5,000 and 10,000 cells per well since they will reach optimal population densities within 48-72 hours.

   Note: For cell types that have 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.

2. Carry out desired cell treatment by adding chemicals or biological agents. Each test should contain a blank containing complete medium without cells (background control, see 8.). The final volume of culture medium in each well should be 100 µL, and the medium may contain up to 10% serum.

3. Defrost the XTT reagent solution and the activation solution immediately prior to use in a 37°C bath. If sediment is present in the XTT solution, heat the solution to 37°C and swirl gently until clear solutions are obtained.

4. To prepare a reaction solution sufficient for one microtiter plate (96 wells), add 25 µl activation solution to 5 ml XTT to derive activated XTT solution. Volumes can be scaled up as necessary.

5. Add 25 µl or 50 µl of the reaction solution to each well and incubate the plate in an incubator for 2-24 hours (usually, 2-5 hours are sufficient). Note: 50 µl activated XTT solution generates a sharper increase of signal but reaches signal saturation at a lower cell number than 25 µl activated XTT solution. 25 µl activated XTT solution gives a broader dynamic range detection. If the volume of 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 uL growth medium).

6. Shake the plate gently to evenly distribute the dye in the wells. Incubate the plate in an incubator for 2-24 hours (usually, 2-5 hours is sufficient).

   Note: 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 points, i.e. after 4, 6, 8, and 12 hours using the same plate.

7. Measure the absorbance of the samples against a background control as a blank with a spectrophotometer (e.g. ELISA reader) at a wavelength of 450-500 nm. Measure background absorbance at a wavelength between 630-690 nm. Subtract background absorbance from signal absorbance to obtain normalized absorbance values.

8. Background control (blank): Slight spontaneous absorbance around 450-500 nm occurs in the culture medium incubated with the XTT reagent. This background absorbance depends on the culture medium, pH, 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. Substract 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 5,000 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.

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 100 µl, add a larger amount of reaction mixture by the same increment (i.e. 100 µl reaction mixture to 200 µl growth media).

Summary of Proliferation Assay Using XTT Reagent: Flow Chart

- Defrost XTT reagent and activation reagent (37°C)
- Prepare reaction mixture (25 µl activation reagent and 5 ml XTT reagent for one 96-well plate)
- Add 25 µl or 50 µl reaction mixture to each well (containing 100 µl media)
- 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 nanometer (reference absorbance: at a wavelength of 630-690 nanometer)

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

References

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* Scudiero, P.A., et al, (1988), Cancer Res. 48, 4827-4833
* Tada, H., et al, (1986), J. Immunol. Meth. 93, 157-165
* Weislow, O.S., et al, (1989), J. Natl Cancer Inst. 81, 577-586

FAQs: PromoKine Cell Viability Kit III (XTT)

- How does the XTT Cell Viability Assay work?

The XTT Cell Viability Assay is a colorimetric assay system which measures the reduction of a tetrazolium component (XTT) into soluble formazan product by the mitochondria of viable cells. The samples are read using an ELISA plate reader at a wavelength of 450nm. The amount of color produced is directly proportional to the number of viable cells.

- What does XTT system does that trypan blue cannot?

The XTT system is a quantitative, more sensitive test. Because there is a linear relationship between cell activity and absorbance, the growth or death rates of cells can be measured. The trypan blue test is qualitative and indicates only if a cell is alive. The XTT assay can also be adapted to high-throughput screening, whereas trypan blue tests must be read individually.

- How long does it take to develop orange formazan color?

We recommend 2 to 4 hours as a starting point. This will vary somewhat with different cell types; there may be some cell types that require up to 24 hours.

- How many cells are required to obtain an efficient reading with the XTT Cell Viability Assay?

For most tumor cells, hybridomas, and fibroblast cell lines, we recommend 5,000 cells per well to perform proliferation assays, although as few as 1,000 cells per well have been used successfully. The known exceptions are blood lymphocytes, which require approximately tenfold more cells (25,000-250,000 cells/well) to obtain a sufficient absorbance reading.

- Are there cell types which will not work with the XTT Cell Viability Assay?

Cells with functional mitochondria are needed to convert the tetrazolium dye in its reduced form. Most eukaryotic cells in culture, including mammalian (suspension and adhesion), plant, and yeast cell types, reduce the dye sufficiently to perform accurate assays. But sometimes keratinocytes and melanocytes will show low metabolic activity.

- How do XTT Cell Viability Assays results compare to (^{3}H)-thymidine incorporation assays?

Because the XTT Cell Viability Assay requires less cell manipulation than (^{3}H)-thymidine incorporation assays (no cell harvesting or medium changes are necessary), the possibility of errors is reduced and the standard deviation values are lower. Comparisons between (^{3}H)-thymidine incorporation and XTT assays have demonstrated less than 5% difference for the determination of growth factor response.

- Can the XTT Cell Viability Assay directly replace (3H)-thymidine incorporation assays?

Yes, the addition of dye solution can be substituted at the point in the assay when radioactive thymidine is added.

- Can the XTT Cell Viability Assay be used for cytotoxicity studies?

Yes, XTT Cell Viability Assay can be used to study cell death mediated by a cytotoxic agent.

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