CELL VIABILITY

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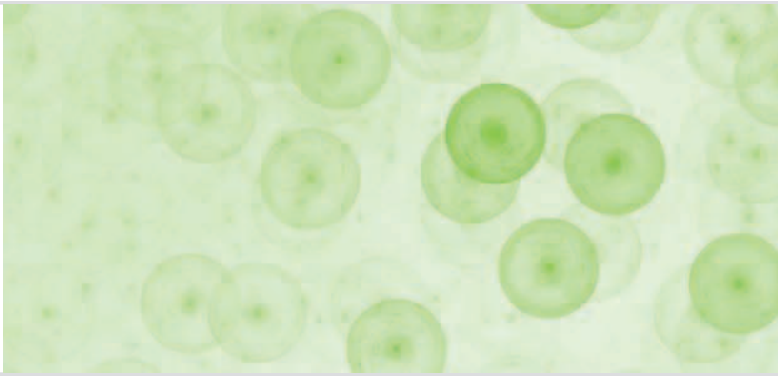


Cell Proliferation Kit (XTT Based)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Cell Proliferation Kit (XTT based)	20-300-1000	1000 assays	-20°C

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, 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 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 51Cr 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

- 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.
- 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

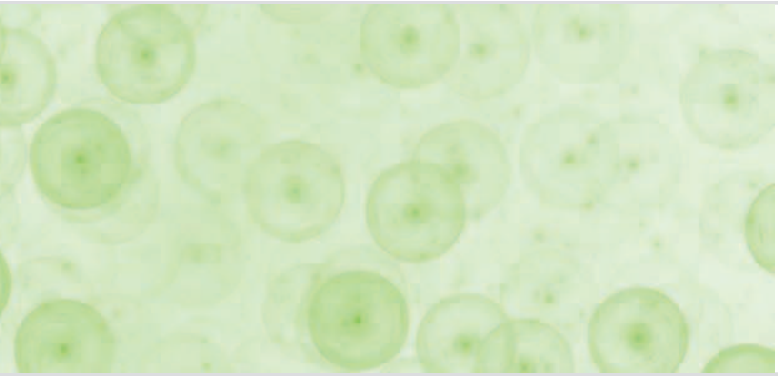
The assay is based on the ability of metabolic active cells to reduce the tetrazolium salt XTT to orange colored compounds of formazan. The dye formed is water soluble and the dye intensity can be read at a given wavelength with a spectrophotometer. The intensity of the dye is proportional to the number 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, 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.

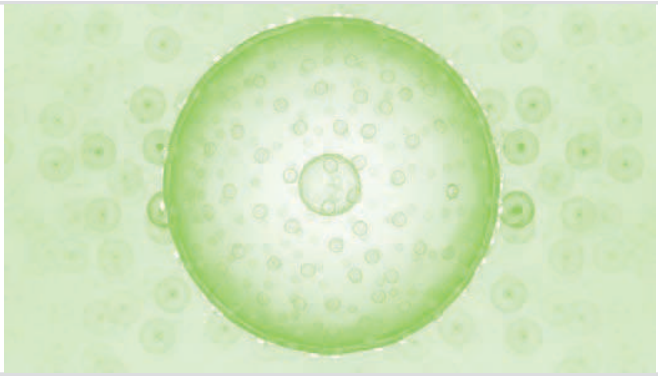
Typical Experiment: The Cytotoxicity of Butylated Hydroxyanisole (BHA)

Butylated Hydroxyanisole (BHA)- synthetic antioxidant used in the food and cosmetic industry.

Mechanism of cytotoxicity

Low doses of BHA exerted a significant cytotoxic effect, associated with loss of mitochondrial function. As the concentration of BHA increases, morphological alterations in critical sub-cellular targets such as lysosomes, mitochondria and actin cytoskeleton, are observed. In parallel, BHA induced an irreversible loss of cell proliferative capacity, preceding apoptosis induction.





The cytotoxic system
Vero cells were exposed to increased concentrations of BHA (0-500µM) for 24 hours to create a cytotoxic system.

BHA cytotoxicity of Vero cells
Vero cells were cultured (5000 cells per well) in a 96 well plates for 24 hours. Cells were exposed to increased concentrations of BHA (0-500µM) for 24 hours, then viability was measured, using a colorimetric method (XTT Based Cell Proliferation Kit , Cat. No. 20-300-1000). XTT reagent was added and absorbance was measured (wavelength of 450nm and reference of 690nm) after a further 5 hours of incubation.

Figure 1: Determination of the cytotoxicity activity of BHA on vero cells.

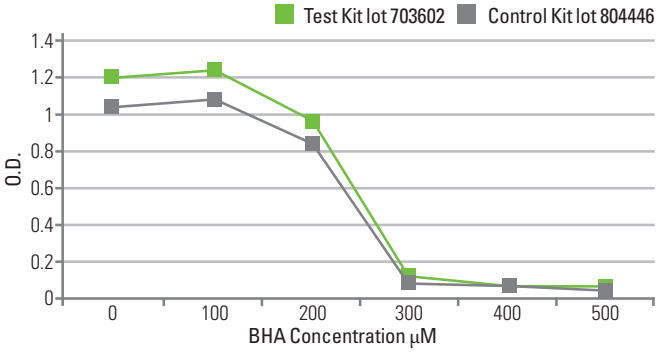
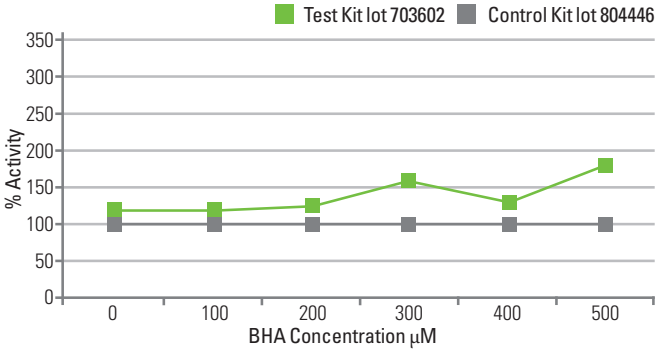


Figure 2: % Activity using test and control kit.
Each point of the control kit was defined as 100% activity. The % activity of the test kit is presented as percentage from the control kit at the same point.



Trypan Blue (0.5% Solution)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Trypan Blue Solution 5mg/ml in Saline	03-102-1B	100ml	AMB

Trypan Blue is the stain most commonly used to distinguish viable from nonviable cells. Viable cells exclude the dye, while nonviable cells absorb the dye and appear blue. Cells should be in suspension as single cells in buffered saline before counting.
Trypan Blue has a higher affinity for serum protein than for cellular proteins, so suspending cells in medium containing serum will generate a dark background.