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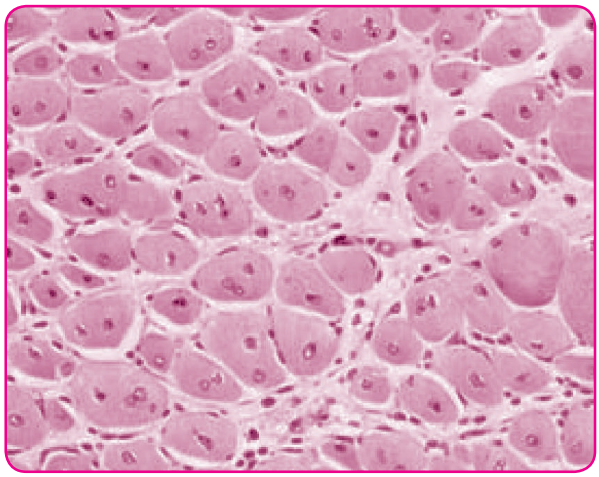
Cell Proliferation Assay Kit XTT

Introduction

Cell proliferation assays are widely used in cell biology for the study of growth factors, cytokines or media components. They are also applied in the screening of cytotoxic agents and lymphocyte activation in order to determine the number of viable cells.

Cell Proliferation Kit XTT employs 2,3-Bis-(2-methoxy 4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT). Only in living cells mitochondria are capable to reduce XTT to form an orange colored water soluble dye. Therefore, the concentration of the dye is proportional to the number of metabolically active cells.



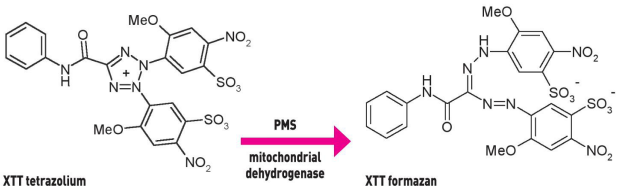
Principle of the XTT reduction salt

The use of tetrazolium salts, such as MTT, commenced in the 1950s, is based on the fact that living 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 an insoluble formazan compound which required 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.

Keywords

- XTT assay
- Cytotoxicity testing
- Non radioactive assay
- Quantification of viable cells



The tetrazolium salt of XTT (2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide salt) is an inner salt, that is cleaved to formazan by the succinate dehydrogenase system of the mitochondrial respiratory chain. Only living cells, possessing an intact mitochondrial membrane and also an intact cell membrane, do have active dehydrogenase. Agents that disrupt the membranes and destroy the respiratory chain will inactivate the enzyme and therefore the formation of the soluble orange formazan by reduction of the yellow tetrazolium salt. The reaction requires the presence of an electron coupling reagent, which is phenazine methosulfate, serving as an intermediate electron acceptor.

XTT Assay Greatly Simplifies Measuring of Cell Proliferation and Viability

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 (usually within 2 – 5 hours) an orange color is formed, the intensity of which can be measured with a spectrophotometer, in this instance, an ELISA reader. The intensity of the dye is proportional to the number of metabolically active cells, i.e. the greater the number of metabolically active cells in the well, the greater the activity of mitochondrial enzymes, and the higher the concentration of the dye formed. The dye formed is water soluble and the dye intensity can be read without further treatments. The use of multiwell plates and an ELISA reader enables testing a large number of samples and obtaining rapid results.

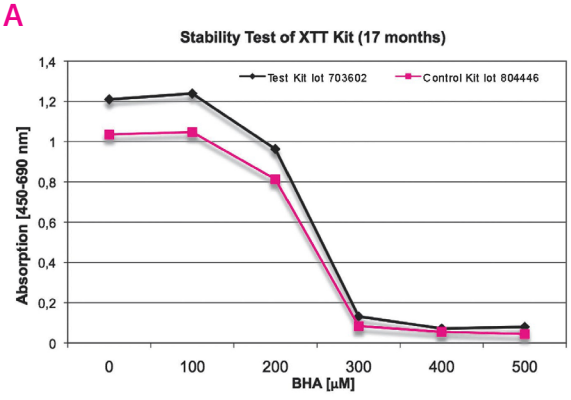
Main Advantages

- **Safe:** without using radioactive isotopes
- **Accurate:** dye absorbance is proportional to the number of cells in each well
- **Easy-to-use:** 1-step process, results within 2 – 5 hours.
- Includes **XTT reagent and activation reagent.** Additional reagents or cellwashing procedures are not required.
- For use in plate readers.

Stability Test of the Cell Proliferation Kit XTT: The Cytotoxicity of Butylated Hydroxyanisole (BHA)

To evaluate the stability of the Cell Proliferation Kit XTT reagents, tests were performed with kits up to 20 months after date of production, using the same lot 703602 in comparison to new standard kits. Graphs show samples tested at points of 17 and 20 months, respectively. Vero cells were cultured in 96 well plates for 24 h. Then, cells were exposed to increasing concentrations of BHA for 24 h, then viability was measured, using the colorimetric Cell Proliferation Kit XTT based method.

A



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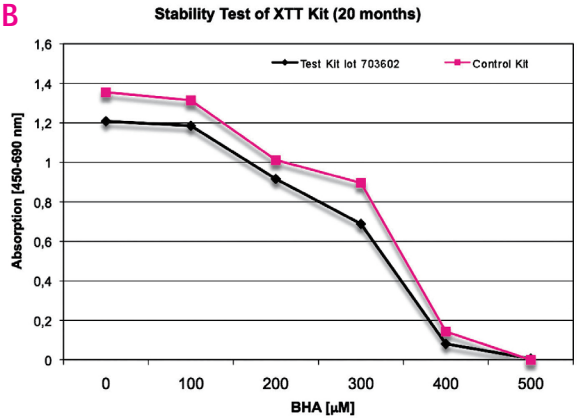

Vero cells were cultured (5000 cells per well) in a 96 well plate for 24 h. Then, cells were exposed to increasing concentrations of BHA (0 – 500 µM) for 24 h, then viability was measured, using the colorimetric Cell Proliferation Kit XTT based method. XTT reagent was added and absorbance was measured (wavelength of 450 nm and reference of 690 nm) after a further 5 h of incubation. The graphs show two representative experiments.

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.

Summarised procedure to use the Cell Proliferation Assay Kit XTT

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

Kit components:

XTT Reagent (10 x 5 ml)
Activation Reagent (2 x 0.5 ml) containing PMS (N-methyl dibenzopyrazine methyl sulfate)

Storage and stability

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.

List of cells which were tested with Cell Proliferation Kit XTT

Pancreatic carcinoma cell line
Monocytes
Human hepatocarcinoma cells – Hep G2
Ehd-1 embryonic fibroblasts
Human embryonic 293 kidney cells
Mouse fibroblasts
Human prostate carcinoma cells – CL1, 22RV1 (subclone of CWR22 xenograft) and LNCaP
Myofibroblasts
Prostate cancer cell lines DU-145 and PC-3
Mammary gland breast cancer cell lines MCF-7alpha and MDA-MB231
Epithelial colorectal adenocarcinoma cell line HT-29
Small cell lung carcinoma cell line AL-780
Mouse myeloid cell line
Primary human umbilical vein endothelial cells
ALL cells of B cell lineage
FDCP cell line
Human monocytic cell lines U937, THP-1 and monomact
Mouse macrophage like cell line RAW 264.7
Freshly isolated human T cells isolated into CD3, CD4 and CD28 populations
293T – (embryonic kidney)
Hela – (cervix carcinoma)
Hep G2 – (heptocellular carcinoma)
D 145 – (prostate cancer)
A375 – (malignant melanoma)
MCF-7 – (breast adenocarcinoma)
BXPC-3 – (pancreatic adenocarcinoma)
Synovial cells
CD3 T-cells from 4–6 week mouse spleens
Human keratinocytes (HaCat) and murine fibroblasts (NIH 3T3)
Human breast cancer cells (T-47D, MDA-MB-468)
Murine C3H10T1/2 progenitor cells
Human embryonic kidney cells (HEK 293)
CHO cells
Early and late murine hematopoietic cells

IP-029EN;201603

Ordering information

Description	Code	Package
Cell Proliferation Kit XTT **Kit components:** XTT Reagent -- 10 x 5 ml Activation Reagent * -- 2 x 0.5 ml * containing PMS (N-methyl dibenzopyrazine methyl sulfate)	A8088,1000	for 1000 tests

Related Products

Description	Code	Package
XTT Sodium Salt BioChemica	A2240,0050	50 mg
	A2240,0100	100 mg
	A2240,0500	500 mg
Dimethyl Sulfoxide Cell culture grade	A3672,0050	50 ml
	A3672,0100	100 ml
	A3672,0250	250 ml
Dimethyl Sulfoxide, sterile filtered (glass ampoule)	A7248,0005	5x5 ml
	A7248,0010	5x10 ml
Incubator-Clean™	A5230,0500	500 ml
	A5230,1000	1 L
	A5230,5000RF	5 L

References:

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