

XTT Cell Viability Kit

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For Research Use Only. Not for Use in Diagnostic Procedures.

Description: The XTT Cell Viability Assay Kit is a colorimetric assay that detects the cellular metabolic activities. During the assay, the yellow tetrazolium salt XTT is reduced to a highly colored formazan dye by dehydrogenase enzymes in metabolically active cells. This conversion only occurs in viable cells and thus, the amount of the formazan produced is proportional to viable cells in the sample. The formazan dye formed in the assay is soluble in aqueous solution and can be quantified by measuring the absorbance at wavelength 450 nm using a spectrophotometer. An electron coupling reagent, such as PMS (N-Methylphenazonium methyl sulfate), can significantly improve the efficiency of XTT reduction in cells.

Background: Cell viability and proliferation assays are widely used in drug discovery for the study of growth factors, cytokines, and cytotoxic agents. High throughput screening, in early drug discovery compound screening and in later drug safety and toxicity studies, requires a reliable, sensitive, and simple assay with the ability to analyze a large number of samples. Colorimetric cell viability assays using tetrazolium salt, such as MTT, XTT, WST-1, etc. were developed based on live cells reduction of tetrazolium salt into highly colored formazan compounds (1,2). In contrast to cell proliferation assays, such as radioactive thymidine or BrdU labeling of DNA in live cells followed by quantification of the incorporated thymidine (by quantifying sample radioactivity) or BrdU (using anti-BrdU antibody), the XTT assay doesn't require radioactive materials, cell fixing, or cell permeabilization. Thus, unlike alternative cellular analysis assays, cells examined in the XTT assay may be used for further analysis.

Specificity/Sensitivity: The XTT Cell Viability Kit detects formazan dye produced from XTT conversion by mitochondrial enzymes in cells. Because these mitochondrial enzymes are inactivated shortly after cell death, the orange colored formazan dye only appears in viable cells. This XTT Cell Viability Kit is expected to work in most cells lines. Variable with cell type and incubation time employed in the assay, $0.2\text{--}2 \times 10^4$ cells/well should be sufficient for most experiments. For the best result, a cell number titration (as shown in Figure 1) is recommended.

Storage: The components within the XTT Cell Viability Kit are stable for 36 months at -20°C.

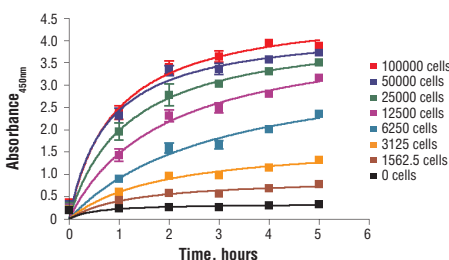
Background References:

- Scudiero, D.A. et al. (1988) *Cancer Res* 48, 4827-33.
- Roehm, N.W. et al. (1991) *J Immunol Methods* 142, 257-65.

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| Product Includes | Item # | Kit Quantity | Color |
|----------------------------|--------|--------------|--------|
| XTT Reagent | 6905 | 2 x 25 ml | |
| Electron Coupling Solution | 9096 | 2 x 0.5 ml | Yellow |

Note: All components in this kit are stable for at least 36 months when stored at the recommended temperature and left unused.



◀ Figure 1. C2C12 cells were seeded at varying density in a 96-well plate and incubated overnight. The XTT assay solution was added to the plate and cells were incubated. The absorbance at 450 nm was measured at 1.0, 2.0, 3.0, 4.0, and 5.0 hours.

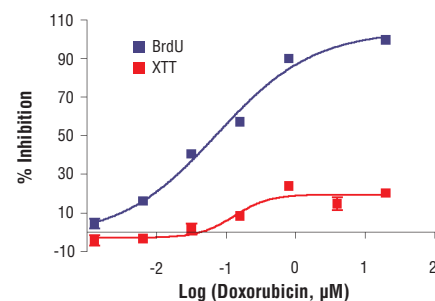
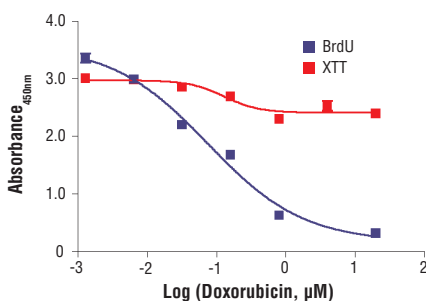


Figure 2. C2C12 cells were seeded at 2×10^4 cells/well in a 96-well plate and incubated overnight. Cells were then treated with various concentrations of doxorubicin overnight. The cytotoxicity was measured using XTT Cell Viability Kit (red) and BrdU Cell Proliferation Assay Kit #6813 (blue) as shown in the left panel. The percentage inhibition in each assay was calculated and plotted in the right panel. Doxorubicin treatment can lead to cell DNA damage followed by cell cycle arrest.

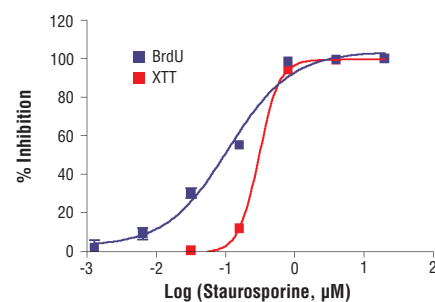
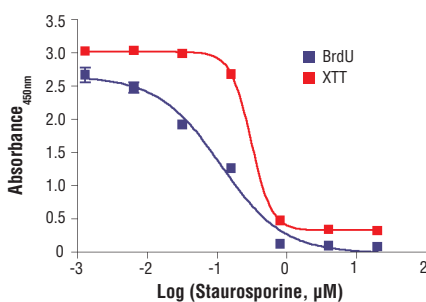


Figure 3. HeLa cells were seeded at 1×10^4 cells/well in a 96-well plate and incubated overnight. Cells were then treated with various concentrations of staurosporine overnight. The cytotoxicity was measured using XTT Cell Viability Kit (red) and BrdU Cell Proliferation Assay Kit #6813 (blue) as shown in the left panel. The percentage inhibition in each assay was calculated and plotted in the right panel. Staurosporine is a nonspecific kinase inhibitor and induces cellular apoptosis.

Assay protocol

A. Reagent Preparation

1. Thaw reagents just before experiment.
Note: Precipitation may occur when reagents are stored at -20°C. Make sure reagents are clear prior to use. If necessary, warm reagents to 37°C to reconstitute.
2. Add electron coupling solution to XTT Reagent (1:50 volume ratio) to make XTT detection solution. For example, each 96-well plate needs 5 ml XTT solution and 0.1 ml electron coupling solution.

B. XTT Assay

1. Add 50 μ l XTT detection solution to each well of 96-well plate (which contains 100-200 μ l/well culture medium) and return plate to incubator.
2. Read absorbance at 450 nm.
Note: The optimal incubation time for this assay depends on experimental setup, such as: cell type, cell number, and treatment. Optimization of incubation time can be determined by reading one plate at various time points after addition of XTT detection solution (as shown in Figure 1).