

QMTT Cell Viability Assay Kits

Non-radioactive Colorimetric Assay for Cell Proliferation and Cytotoxicity

DESCRIPTION

The study of cell proliferation and cell viability requires the accurate quantification of the number of viable cells in a cell culture. Therefore, assays for calculating cell viability are necessary for optimizing cell culture conditions, evaluating cell growth factors and nutrients, discovering novel antibiotics and anti-cancer drugs, evaluating toxic effects of environmental pollutants and cell mediated toxicity and studying programmed cell death (apoptosis).

The QMTT assay kit provides a convenient, sensitive, quantitative and reliable assay for determining the number of viable cells in a given culture. This homogeneous colorimetric assay is based on the conversion of a tetrazolium salt MTT, a pale yellow substrate, to formazan, a purple dye. This cellular reduction reaction involves the pyridine nucleotide cofactors NADH/NADPH and is only catalyzed by living cells. The formazan product has a low aqueous solubility and is present as purple crystals. Dissolving the resulting formazan with a solubilization buffer permits the convenient quantification of product formation. The intensity of the product color, measured at 550 - 620 nm, is directly proportional to the number of living cells in the culture. Reagents in the kit have been carefully formulated and optimized for sensitivity, assay robustness and automation.

KEY FEATURES

Safe. Non-radioactive assay (cf. ³H-thymidine incorporation assay).

Sensitive and accurate. As low as 950 cells can be accurately quantified.

Fast. High-throughput assay using 96-well plates allows simultaneous processing tens of thousands of samples per day.

Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Robust and amenable to HTS. Z' factors of 0.5 and above are observed. Can be readily automated with HTS liquid handling systems.

APPLICATIONS

Cell Proliferation: effects of cytokines, growth factor, and nutrients.

Cytotoxicity and Apoptosis: evaluation of toxic compounds, anti-cancer antibodies, toxins, environmental pollutants etc.

Drug Discovery: high-throughput screen for toxic and anticancer drugs.

KIT CONTENTS

Catalog #	Size (assays)	Reagent	Assay Buffer	Solubilizer
Z5030006	500	solid	10 mL	50 mL
Z5030007	1,000	solid	20 mL	100 mL

Control Reagent (Cat # Z5030008): 50 mg saponin (to be ordered separately).

Storage conditions. Store the Reagent at -20 °C. Keep Assay Buffer and Solubilization Solution at 4 °C and room temperature, respectively. Shelf life: 12 month.

This protocol can be downloaded online at www.biochain.com.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

The QMTT assay is based on the conversion of MTT to purple formazan by metabolically active cells. For most cells, this reducing reaction takes 3 to 5 hours. The produced formazan is solubilized and the resulting colored solution is quantified with a microplate reader. Although most culture media contain phenol red, phenol red does not interfere with the assay. All data in Technical Notes were obtained in culture media containing phenol red.

Procedure using 96-well plate:

1. Plate and culture cells (80 µL per well) in a clear bottom 96-well tissue

culture plates. Typical culture medium contains DMEM, 10% fetal bovine serum, antibiotics (penicillin/streptomycin, gentamycin, etc), amino acids and other nutrients. Assays can be performed on either adherent cells or cells in suspension. The number of cells can vary from 1,000 to 80,000 per well. The volume can vary from 50 to 150 µL, although 80 µL is used in this example. In addition to the test samples, one must include control wells of culture medium containing no cells or cells treated with a toxic reagent such as 0.1% saponin.

2. Add test compounds and controls and incubate cells for the desired period of time (typically overnight). It is recommended that assays be run in duplicate or triplicate. A volume of 20 µL in phosphate buffered saline (PBS) or culture medium is recommended for the test compounds and controls. The Control reagent can be conveniently reconstituted with 5 mL PBS (1% saponin).

3. Reconstitute the QMTT Reagent with Assay Buffer. First equilibrate the Reagent and Assay Buffer to room temperature. Then simply combine Assay Buffer and the Reagent by pipetting a small volume (e.g. 1 mL) buffer to the Reagent tube. Vortex briefly and pipet the reconstituted solution to the Assay Buffer bottle. Repeat this step to transfer all reagent to the Assay Buffer bottle. Mix by inversion until the Reagent is thoroughly dissolved. After this is done, mark the bottle label as Reconstituted Reagent. Note: Fresh reconstitution is recommended although the reconstituted reagent is stable for up to 4 weeks when stored at -20 °C.

4. Add 15 µL (per 80 µL cell culture) of QMTT reagent per well and incubate for 4 hours at 37°C. The volume of the reagent should be adjusted depending on the volume of cell culture.

5. Add 100 µL of the Solubilization Solution. Mix gently on an orbital shaker for one hour at room temperature. The volume of the Solubilization Solution should be adjusted depending on the volume of cell culture. If precipitation occurs in the Solubilization Solution, place the bottle in a warm water bath or at 37°C and shake to dissolve precipitates.

6. Measure OD_{570nm} for each well on an absorbance plate reader. Maximum absorbance of the formazan dye lies between 560 and 590 nm. If desired, the OD measurement can be performed the following day. In this case, it is recommended to seal the plate to minimize evaporation.

DATA ANALYSIS

Determine the average of the blank controls and subtract this amount from all absorbance values. Plot the corrected absorbance values at 570nm against the concentration of the test compound. Determine the EC₅₀ value for cell proliferation and IC₅₀ value for cytotoxic compound by non-linear regression analysis using Prism or any other data analysis tools.

LITERATURE

Cell proliferation:

1. Luan X, Diekwisch TG (2002). CP27 affects viability, proliferation, attachment and gene expression in embryonic fibroblasts. *Cell Prolif.* 35: 207-19.

Cytotoxicity assays

2. Bezivin C, Tomasi S, Lohezic-Le Devehat F, Boustie J (2003). Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomedicine.* 10(6-7):499-503.

3. Koh J, Kubota T, Koyama T, Migita T, Hashimoto M, Hosoda Y, Kitajima M (2000). Combined Antitumor Activity of 7-Hydroxystaurosporine (UCN-01) and Tamoxifen against Human Breast Carcinoma in vitro and in vivo. *Breast Cancer.* 10(3):260-7.

4. Labieniec M, Gabryelak T (2003). Effects of tannins on Chinese hamster cell line B14. *Mutat Res.* 539(1-2):127-35.

5. Camps J, About I (2003). Cytotoxicity testing of endodontic sealers: a new method. *J Endod.* 29(9):583-6.

QMTT Cell Viability Assay Kits Non-Radioactive Colorimetric Assay for Cell Proliferation and Cytotoxicity

6. Di Francesco AM, Mayalarp SP, Kim S, Butler J, Lee M (2003). Synthesis and biological evaluation of novel diaziridinylquinone-acridine conjugates. *Anticancer Drugs*. 14(8):601-15.

7. Moongkarndi P, Kaslungka S, Kosem N, Junnu S, Jongsomboonkusol S, Theptaranon Y, Neungton N (2003). Cytotoxicity and apoptosis of ovarian and breast cancer cell lines induced by OVS1 monoclonal antibody and paclitaxel. *Asian Pac J Allergy Immunol*. 21(1):31-41.

8. Yamashita T, Fujii M, Tomita T, Ishiguro R, Tashiro M, Tokumaru Y, Imanishi Y, Kanke M, Ogawa K, Kameyama K, Otani Y (2003). The inhibitory effect of matrix metalloproteinase inhibitor ONO-4817 on lymph node metastasis in tongue carcinoma. *Anticancer Res*. 23(3B):2297-302.

in vitro Chemosensitivity

9. Nakamori M, Iwahashi M, Nakamura M, Yamaue H(2003). Clinical benefit of chemosensitivity test for patients with regional lymph node-positive esophageal squamous cell carcinoma. *J Surg Oncol*. 84(1):10-6.

10. Ariyoshi Y, Shimahara M, Tanigawa N(2003). Study on chemosensitivity of oral squamous cell carcinomas by histoculture drug response assay. *Oral Oncol*. 39(7):701-7.

11. Yoshinare K, Kubota T, Watanabe M, Wada N, Nishibori H, Hasegawa H, Kitajima M, Takechi T, Fukushima M(2003). Gene expression in colorectal cancer and in vitro chemosensitivity to 5-fluorouracil: A study of 88 surgical specimens. *Cancer Sci*. 94(7):633-638.

12. Tallen G, Riabowol K, Wolff JE(2003). Expression of p33ING1 mRNA and chemosensitivity in brain tumor cells. *Anticancer Res*. 23(2B):1631-5.

Screening for cytotoxic compounds

13. Nishimura S, Murasugi T, Kubo T, Kaneko I, Meguro M, Marumoto S, Kogen H, Koyama K, Oda T, Nakagami Y(2003). RS-4252 inhibits amyloid beta-induced cytotoxicity in HeLa cells. *Pharmacol Toxicol*. 93(1):29-32.

14. Nakagami Y, Nishimura S, Murasugi T, Kubo T, Kaneko I, Meguro M, Marumoto S, Kogen H, Koyama K, Oda T(2002). A novel compound RS-0466 reverses beta-amyloid-induced cytotoxicity through the Akt signaling pathway in vitro. *Eur J Pharmacol*. 457(1):11-7.

15. Wu J, Zhang H, Wang J, Yang T, Xian J, Yang C, Zheng W, Chen H, Wang Q(2002). Screening for inhibitor of vascular endothelial growth factor from random peptide library. *Zhonghua Zhong Liu Za Zhi*. 24(6):540-3.

16. Ren DC, Du GH, Zhang JT(2003). High throughput screening for intercellular adhesion molecule-1 inhibitor. *Yao Xue Xue Bao*. 38(6):405-8.

TECHNICAL NOTES

The QMTT assay kit provides a convenient and homogeneous assay for cell proliferation and cytotoxicity in multi-well plates. Reagents of the kit have been carefully formulated and optimized for sensitivity, assay robustness and automation. Key features of the kits include:

Safe. Non-radioactive assay (cf. ^3H -thymidine incorporation assay).

Sensitive and accurate. As low as 950 cells can be accurately quantified.

Fast. High-throughput assay using 96-well plates allows simultaneous processing tens of thousands of samples per day.

Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Robust and amenable to HTS: Z' factors of 0.5 and above are observed. Can be readily automated with HTS liquid handling systems.

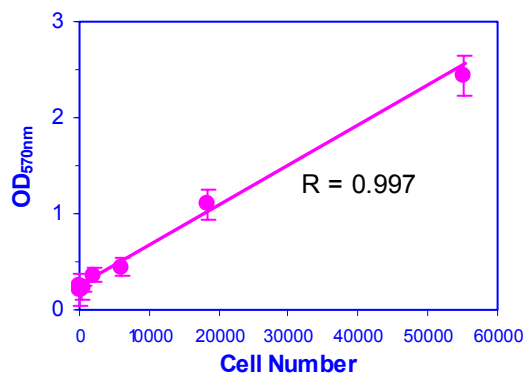


Figure 1. HEK293 cells were plated in clear bottom 96-well plates. Cells were serially diluted from 55,000 cells to 25 cells in 100 μL DMEM plus 10% FBS. 15 μL of the QMTT reagent was added and cells were incubated for 4 hours at 37°C. Then 100 μL of the Solubilization Solution was added and the plate was shaken for 1 hour at room temperature. The OD_{570nm} (cm^{-1}) was measured on a Molecular Devices SpectraMax 384 Plus reader. A linear relationship was observed between OD_{570nm} and the cell number. The detection limit was estimated to be 950 cells from the blank control.

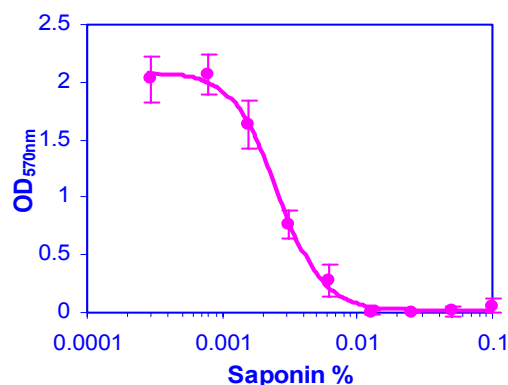


Figure 2. Cytotoxic effects of saponin in HEK293 cells. HEK293 cells were plated at 55,000 cells per 80 μL well in clear bottom 96-well plate. Cells were treated with varying concentrations of saponin. After overnight incubation, 15 μL of QMTT reagent was added and further incubated for 4 hours. 100 μL of the Solubilization Solution was then added. The plate was shaken for 1 hour at room temperature and OD_{570nm} (cm^{-1}) was read on a Molecular Devices SpectraMax 384 Plus reader. The IC₅₀ for saponin was 0.0026 wt%.