CyQUANT™ MTT Cell Proliferation Assay Kit

Catalog Numbers V13154

Pub. No. MAN0019028 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ CyQUANT™ MTT Cell Proliferation Assay Kit (Cat. No. V13154) provides a simple method to measure cellular proliferation and viability using a microplate reader. Determination of cell growth rates is widely used for drug cytotoxicity testing, as well as screening of other biologically-active compounds. Several methods can be used, however, fluorescent or chromogenic indicators provide a rapid, cost-effective method for determining changes to cell viability.

The MTT assay, developed by Mossman (Mosmann, 1983), is one of the most versatile and well-established assays to determine cell viability. The redox potential in viable mammalian cells causes the conversion of water-soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to insoluble formazan (Liu, 1997; Berridge, 1997; Vistica, 1991). Formazan is then solubilized, and the concentration of the colorimetric probe is determined by measuring optical density at 570 nm. The result is a sensitive assay with linearity up to approximately 10^6 cells per well (Figure 1).

This document provides guidance for the complete assay protocol that requires an overnight incubation, and a rapid protocol that can be performed in five hours (not including cell preparation time). For additional information concerning variations and modifications of the MTT assay, consult the citations provided (Garn, 1994; Carmichael, 1987; Twentyman, 1987; Tada, 1986).

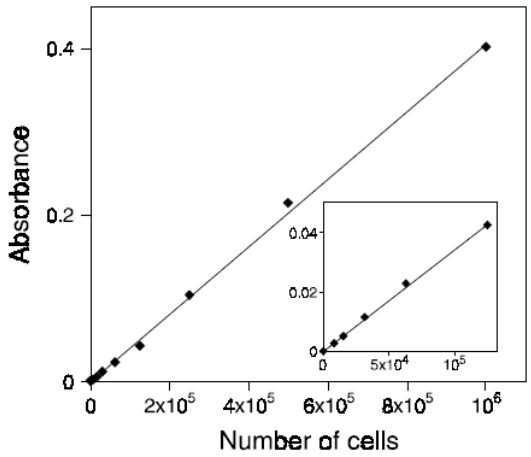

Figure 1 Quantitation of Jurkat cells using the CyQUANT™ MTT Cell Proliferation Assay Kit

Cells in the parent culture were counted, then diluted to the indicated densities in 100-µL volumes. The cells were then transferred to wells of a microplate and incubated for 4 hours to allow time for adsorption. Absorbance was measured at 570 nm using a microplate reader. Each data point represents the mean value of samples in triplicate. The inset shows the data plotted for the lower cell numbers.

Contents and storage

Reagents that are provided in the kit are sufficient for 1,000 individual tests using standard 96-well microplates.

Contents	Amount	Storage[1]
MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MW = 414 (Component A)	10 × 5 mg[2]	4°C, protect from light
SDS sodium dodecyl sulfate, MW = 288 (Component B)	10 × 1 mg[3]	

[1] See label for expiration date.
[2] Each 5-mg vial of MTT provides sufficient reagent for 100 tests, using 10 µL of the stock solution per well.
[3] Each 1-mg vial of SDS provides sufficient solution for 100 tests, using 100 µL per well.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Phosphate-buffered saline (PBS), sterile	MLS
HCl, 0.01 M solution	MLS
(Optional) Dimethylsulfoxide (DMSO)	MLS

Guidelines for culturing cells

Cell culture conditions can affect the results of the assay and must be taken into consideration when analyzing the data. The age of cell cultures, number of passages, and details of the growth medium can all be important factors. Natural variation in the requirements and growth rates of different cell lines make it difficult to provide precise guidelines for cell preparation. As a starting point, we recommend seeding cells at a density between 5,000–10,000 cells per well to reach optimal population densities within 48–72 hours.

IMPORTANT! The presence of phenol red can affect MTT assay results. We strongly recommend that cells are cultured in medium without phenol red. If needed, transfer cells to a medium without phenol red before the final incubation with MTT.

Before you begin

On the day of the experiment, prepare the following reagents.

1. Prepare a 12-mM MTT stock solution by adding 1 mL of sterile PBS to one 5-mg vial of MTT (Component A). Vortex to mix or sonicate the solution until it is dissolved. If some particulate material does not dissolve, remove by filtration or centrifugation.

   Note: MTT stock solution can be stored at 4°C for up to 4 weeks protected from light.

2. Prepare the SDS-HCl solution by adding 10 mL of 0.01 M HCl to one tube containing 1 gm of SDS (Component B). Mix the solution gently by inversion or sonication until the SDS dissolves. Once prepared, use the SDS-HCl solution promptly.

Label cells: Complete assay protocol

1. Replace the culture medium according to your cell type.
   - For adherent cells, remove the medium, then add 100 µL of fresh culture medium.
   - For non-adherent cells, centrifuge the microplate, carefully remove as much medium as possible from the cell pellet, then resuspend the cells in 100 µL of fresh medium.
2. Add 10 µL of the 12-mM MTT stock solution to each well. Include a negative control by adding 10 µL of the MTT stock solution to 100 µL of medium alone.
3. Incubate at 37°C for 4 hours or overnight. For cell densities >100,000 cells per well, the incubation time can be shortened to 2 hours.
4. Add 100 µL of the SDS-HCl solution to each well, then pipet up and down thoroughly to mix.
5. Incubate the microplate at 37°C for 4–18 hours in a humidified chamber. Longer incubations will decrease the sensitivity of the assay (Niks, 1990).
6. Pipet up and down to mix each sample again, then read the absorbance at 570 nm.

Label cells: Rapid protocol

This protocol uses DMSO (not provided) as a solubilizing agent for formazan to shorten the time of the assay (Carmichael, 1987).

1. Replace the culture medium according to your cell type.
   - For adherent cells, remove the medium, then add 100 µL of fresh culture medium.
   - For non-adherent cells, centrifuge the microplate, carefully remove as much medium as possible from the cell pellet, then resuspend the cells in 100 µL of fresh medium.
2. Add 10 µL of the 12-mM MTT stock solution to each well. Include a negative control by adding 10 µL of the MTT stock solution to 100 µL of medium alone.
3. Incubate at 37°C for 2–5 hours.
4. Remove all but 25 µL of medium from the wells. For non-adherent cells, it may be necessary to centrifuge the plates to sediment the cells before removing the medium.
5. Add 50 µL of DMSO to each well, then pipet up and down thoroughly to mix.
6. Incubate at 37°C for 10 minutes.
7. Pipet up and down to mix each sample again, then read the absorbance at 540 nm.

   IMPORTANT! Do not read the absorbance at 570 nm as directed for the complete assay protocol.

Ordering information

The following products are also available. Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Amount	Source
MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide	1 g	M6494

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

References

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Revision history: Pub. No. MAN0019028

Revision	Date	Description
B.0	2 September 2021	• Updated the complete assay protocol to include the option to incubate cells overnight after addition of the MTT stock solution. • Corrected the rapid protocol to include an incubation step after addition of the MTT stock solution.
A.0	2 January 2020	• Converted the legacy document (MP 13154) to the current document template, with associated updates to the publication number, warranty, trademarks, and logos. • Changed the kit name from Vybrant® MTT Cell Proliferation Assay Kit to CyQUANT™ MTT Cell Proliferation Assay Kit.

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