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Vybrant® MTT Cell Proliferation Assay Kit (V-13154)

Quick Facts

Storage upon receipt:
- 4°C
- Protect from light

Introduction

Our Vybrant® MTT Cell Proliferation Assay Kit provides a simple method for determination of cell number using standard microplate absorbance readers. Determination of cell growth rates is widely used in the testing of drug action, cytotoxic agents and screening other biologically active compounds. Several methods can be used for such determinations, but indirect approaches using fluorescent or chromogenic indicators provide the most rapid and large scale assays. Among such procedures, the MTT assay developed by Mossman[1] is still among one of the most versatile and popular assays.

The MTT assay involves the conversion of the water soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to an insoluble formazan.[2-4] The formazan is then solubilized, and the concentration determined by optical density at 570 nm. The result is a sensitive assay with excellent linearity up to approximately 10^6 cells per well (Figure 1).

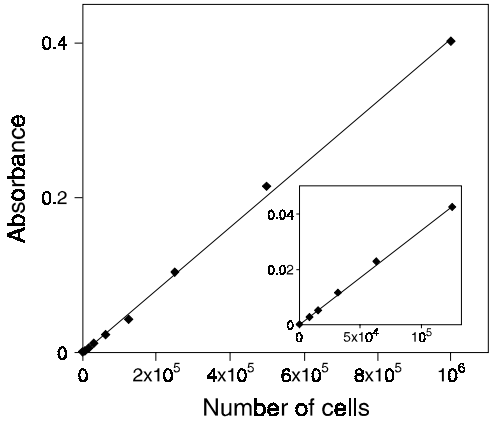

Figure 1. *Quantitation of Jurkat cells using the Vybrant MTT Cell Proliferation Assay Kit. Cells in the parent culture were counted in a hemacytometer and then diluted to the indicated cell numbers in 100 µL volumes, delivered to the wells of a microplate and incubated for 4 hours to allow time for adsorption before being assayed. Absorbance measurements at 570 nm were made 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.*

Our MTT Cell Proliferation Assay Kit provides enough material to perform 1000 individual tests using standard 96-well microplates. Following the protocol described below, a complete assay requires an overnight incubation. However, with a slight modification, the whole procedure can be performed in five hours (not including cell preparation time). For additional information concerning the numerous variations and modifications of the MTT assay, we recommend that you consult the citations provided.[5-8]

Materials

Kit Contents

- **MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide** (MW = 414, Component A), 10 vials, each containing 5 mg
- **SDS sodium dodecyl sulfate** (MW = 288, Component B), 10 vials, each containing 1 gm

Storage and Handling

Upon receipt the kit should be stored at 4°C protected from light. Stored properly, the kit components should remain stable for 12 months.

Materials Required but Not Provided

- phosphate-buffered saline (PBS), sterile
- HCl, 0.01 M solution
- dimethylsulfoxide (DMSO) — optional

Experimental Protocol

Reagent Preparation

1.1 Prepare a 12 mM MTT stock solution by adding 1 mL of sterile PBS to one 5 mg vial of MTT (Component A). Mix by vortexing or sonication until dissolved. Occasionally there may be some particulate material that will not dissolve; this can be removed by filtration or centrifugation. Each 5 mg vial of MTT provides sufficient reagent for 100 tests, using 10 µL of the stock solution per well. Once prepared, the MTT solution can be stored for four weeks at 4°C protected from light.

1.2 Add 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, the solution should be used promptly. Each tube makes sufficient solution for 100 tests, using 100 µL per well.

Culturing Cells

The culture conditions used to grow the cells can affect the results and must be taken into consideration when analyzing the

data. The age of the 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 preparing your cells. In general, cells seeded at densities between 5000–10,000 cells per well should reach optimal population densities within 48–72 hours. Note that the presence of phenol red in the final assay samples can seriously affect results. We strongly recommend that the cells be cultured in medium free of phenol red, if possible. Alternatively, the final incubation with the MTT can be performed after exchanging the cells into medium free of phenol red.

Labeling Cells

2.1 For adherent cells, remove the medium and replace it with 100 µL of fresh culture medium. For non-adherent cells, centrifuge the microplate, pellet the cells, carefully remove as much medium as possible and replace it with 100 µL of fresh medium.

2.2 Add 10 µL of the 12 mM MTT stock solution (prepared in step 1.1) to each well. Include a negative control of 10 µL of the MTT stock solution added to 100 µL of medium alone.

2.3 Incubate at 37°C for 4 hours. At high cell densities (>100,000 cells per well) the incubation time can be shortened to 2 hours.

2.4 Add 100 µL of the SDS-HCl solution (prepared in step 1.2) to each well and mix thoroughly using the pipette.

2.5 Incubate the microplate at 37°C for 4–18 hours in a humidified chamber. Longer incubations will decrease the sensitivity of the assay.[9]

2.6 Mix each sample again using a pipette and read absorbance at 570 nm.

Quick Protocol Option

To shorten the time of the assay it is possible to use DMSO (not provided) as a solubilizing agent to dissolve the formazan.[6]

3.1 After labeling the cells with MTT, as described above, remove all but 25 µL of medium from the wells. For non-adherent cells it may be necessary to first centrifuge the plates to sediment the cells.

3.2 Add 50 µL of DMSO to each well and mix thoroughly with the pipette.

3.3 Incubate at 37°C for 10 minutes.

3.4 Mix each sample again and read absorbance at 540 nm — *not 570 nm*, as above.

References

1. J Immunol Methods 65, 55 (1983); **2.** J Neurochem 69, 581 (1997); **3.** Arch Biochem Biophys 303, 474 (1993); **4.** Cancer Res 51, 2515 (1991); **5.** J Immunol Methods 168, 253 (1994); **6.** Cancer Res 47, 943 (1987); **7.** Br J Cancer 56, 279 (1987); **8.** J Immunol Methods 93, 157 (1986); **9.** J Immunol Methods 130, 149 (1990).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
M-6494	MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)	1 g
V-13154	Vybrant® MTT Cell Proliferation Assay Kit *1000 assays*	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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