For life science research only.
Not for use in diagnostic procedures.

Cell Proliferation Kit I (MTT)

Colorimetric assay (MTT based) for the non-radioactive quantification of cell proliferation and viability

Cat. No. 11 465 007 001

1 Kit (for 2,500 tests)

Version 17
Content version: September 2005

Store at −15 to −25°C

1. Kit contents

Bottle	Label	Contents
1	MTT labeling reagent	▪ 5 vials containing 5 ml MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromid) labeling reagent (1×), ▪ 5 mg/ml in phosphate buffered saline (PBS), ▪ non-sterile ▪ ready to use.
2	Solubilization solution (1×, ready-to-use)	▪ 3 bottles with 90 ml ▪ 10% SDS in 0.01 M HCl.

2. Introduction

2.1 Product overview

Assay principle

The assay is based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by metabolic active cells (Fig. 1) (6, 7, 35).

This cellular reduction involves the pyridine nucleotide cofactors NADH and NADPH (36). The formazan crystals formed are solubilized and the resulting colored solution is quantified using a scanning multiwell spectrophotometer (ELISA reader).

This ensures a high degree of accuracy, enables online computer processing of the data (data collection, calculation and report generation) and, thereby, allows the rapid and convenient handling of a high number of samples.

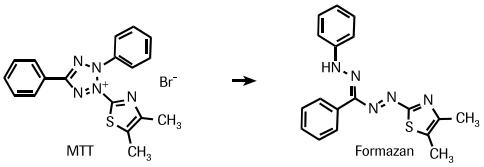

Fig.1: Metabolization of MTT to a formazan salt by viable cells.

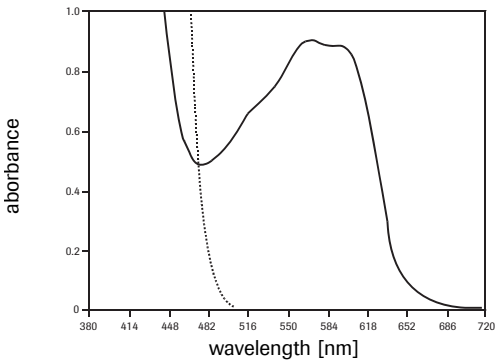

Fig. 2: Comparison of UV-spectra of MTT labeling reagent (dotted line) and the formazan salt after solubilization with solubilization solution.

Basic steps

Stage	Description
1	Cells, grown in a 96 well tissue culture plate, are incubated with the yellow MTT solution for approx. 4 h.
2	After this incubation period, purple formazan salt crystals are formed. These salt crystals are insoluble in aqueous solution, but may be solubilized by adding the solubilization solution and incubating the plates overnight in humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2).
3	The solubilized formazan product is spectrophotometrically quantified using an ELISA reader. An increase in number of living cells results in an increase in the total metabolic activity in the sample. This increase directly correlates to the amount of purple formazan crystals formed, as monitored by the absorbance (see Fig. 3).

Background information

The determination of cellular proliferation, viability and activation are key areas in a wide variety of cell biological approaches. The need for sensitive, quantitative, reliable and automated methods led to the development of standard assays. Such an example is based on the capability of the cells to incorporate a radioactively labeled substance ([^{3}H])- thymidine), or to release a radioisotope such as [^{51}Cr] after cell lysis. Alternatively, the incorporation of 5-bromo-2'-deoxyuridine (BrdU)* in place of thymidine is monitored as a parameter for DNA synthesis and cellular proliferation in immunohisto-and cytochemistry, in a cell ELISA and FACS analysis. (kits and reagents for these applications are available from Roche Applied Science). Cell proliferation and viability assays are of particular importance for routine applications. Tetrazolium salts (*e.g.,* MTT, XTT, WST-1) are especially useful for assaying the quantification of viable cells, because they are cleaved to form a formazan dye (Fig. 1; for UV absorbance spectrum, see Fig. 2) only by metabolic active cells.

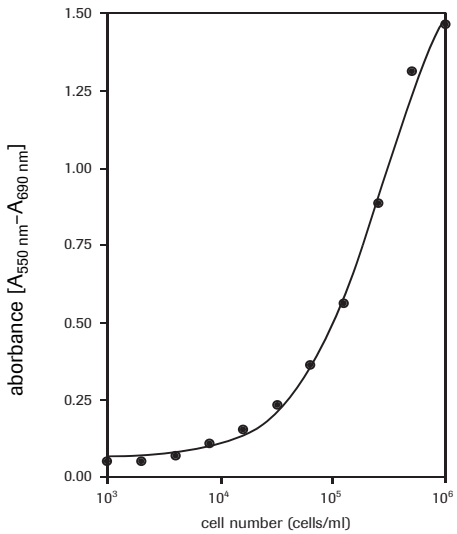

Fig. 3: Effect of different numbers of cells on color formation (example given, using Ag8 cells).

Application

The non-radioactive, colorimetric assay system using MTT was first described by Mosmann, T. *et al.* (1) and improved in subsequent years by several other investigators (2–6).

The assay is designed for the spectrophotometric quantification of cell growth and viability (1, 3, 5–7) without the use of radioactive isotopes.

- It is used for the measurement of cell proliferation in response to growth factors, cytokines and nutrients (1–3, 6, 8–12) (see fig. 4).
- The MTT assay is also useful for the measurement of cytotoxicity. Examples are the quantification of tumor necrosis factor-a or -b effects (13, 14). (see fig. 5) or macrophage induced cell death (15, 16) and the assessment of cytotoxic (17–34) or growth inhibiting agents such as inhibitory antibodies (see fig. 6).
- For the replacement of the radioactive [^{51}Cr]-release cytotoxicity assay, protocols using MTT have been developed. The MTT assay is as sensitive as the radioactive method, but shows a significantly lower background especially after long term incubation (34).
- The MTT assay can also be used to study cell activation (4).

Storage and stability

Stable at -15 to -25°C until the expiration date printed on the label.

Note: Protect from light. Repeated thaw-freeze cycles do not affect product stability. Precipitates may form during shipment or storage, in which case the container should be warmed to +37°C and thoroughly mixed.

After thawing, the MTT labeling reagents may be stored protected from light at +2 to +8°C for up to 4 weeks, in which case a sterile filtration of the reagent is recommended.

Advantages

Compared to radioactive isotope techniques, the Cell Proliferation Kit I (MTT) shows the following benefits.

Benefit	Feature
Safe	No radioactive isotopes are used.
Accurate	The absorbance revealed, strongly correlates to the cell number, (see fig. 3).
Sensitive	Low cell numbers are detected (see fig. 3).
Fast	The use of multiwell-ELISA readers allows for processing a large number of samples.
Easy	No washing steps and no additional reagents are required.

3. Protocols and required material

3.1 Assay procedure

Overview

Please refer to the following table.

Step	Description	Volume/ well	Time/ Temp
	Perform tissue culture using 96 well microplates (tissue culture grade, flat-bottom)	100 µl	24-96 h +37°C.
1	Add MTT labeling reagent and incubate in a humidified atmosphere	10 µl	4 h +37°C.
2	Add solubilization solution and incubate in a humidified atmosphere	100 µl	overnight +37°C
3	Evaluate microplate with the use of an ELISA reader at 550–600 nm with a reference wavelength of >650 nm.		

Handling instruction for larger volumes

If for the initial incubation of the cells a larger volume of culture medium is required, increase the amount of MTT labeling reagent correspondingly (*e.g.,* 20 µl MTT labeling reagent, when cells are cultured in 200 µl culture medium).

Protocol

Please refer to the following table.

Note: If for the initial incubation of the cells a larger volume of culture medium is required, increase then amount of MTT labeling reagent correspondingly (*e.g.,* 20 µl MTT labeling reagent, when cells are cultured in 200 µl culture medium).

Step	Action
1	Cells are grown in microplates (tissue culture grade, 96 wells, flat bottom) in a final volume of 100 µl culture medium per well, according to the media needs of the cells, in a humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2). The incubation period of the cell cultures depends on the particular experimental approach and on the cell line used for the assay. For most experimental setups, the incubation of cells for 24 to 96 h is appropriate.
2	After the incubation period, add 10 µl of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.
3	Incubate the microplate for 4 h in a humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2).
4	Add 100 µl of the Solubilization solution into each well.
5	Allow the plate to stand overnight in the incubator in a humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2).
6	Check for complete solubilization of the purple formazan crystals and measure the spectrophotometrical absorbance of the samples using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 550 and 600 nm according to the filters available for the ELISA reader, used. The reference wavelength should be more than 650 nm.

3.2 Examples

3.2.1. Cell growth assay procedure

Additional reagents required

- Culture medium, *e.g.,* DMEM containing 10% heat inactivated FCS (fetal calf serum), 2 mM glutamine, 0.55 mM L-arginine, 0.24 mM L-asparagine-monohydrate, 50 µM 2-mercaptoethanol, HT-media supplement (1×), containing 0.1 mM hypoxanthine and 16 µM thymidine. If an antibiotic is to be used, additionally supplement media with penicillin/streptomycin or gentamicin *
- Interleukin-6, human (hIL-6) (200,000 U/ml, 2 µg/ml) sterile*.

Protocol

For the determination of human interleukin-6 (hIL-6) activity on 7TD1 cells (mouse-mouse hybridoma) (see fig. 4).

Step	Action
1	Seed 7TD1 cells at a concentration of 2 × 10^3 cells/well in 100 µl culture medium containing various amounts of IL-6 [final concentration *e.g.,* 0.1–10 U/ml (0.001–0.1 ng/ml)] into microplates (tissue culture grade, 96 wells, flat bottom).
2	Incubate cell cultures for 4 days at +37°C and 6.5% CO_2.
3	After the incubation period, add 10 µl of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.
4	Incubate the microplate for 4 h in a humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2).
5	Add 100 µl of the Solubilization solution into each well.
6	Allow the plate to stand overnight in the incubator in a humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2).
7	Check for complete solubilization of the purple formazan crystals and measure the spectrophotometrical absorbance of the samples using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 550 and 600 nm according to the filters available for the ELISA reader, used. The reference wavelength should be more than 650 nm.

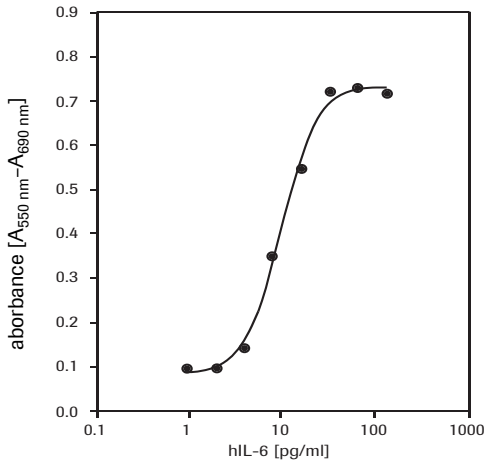

Fig. 4: Proliferation of 7TD1 cells (mouse-mouse hybridoma) in response to recombinant human interleukin-6 (hIL-6) using the procedure described (see section *Examples*, *3.2.1.*).

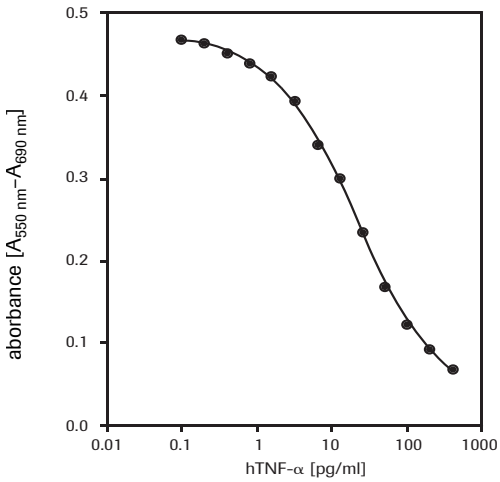

Fig. 5: Determination of the cytotoxic activity of recombinant human TNF-α (h TNF-α) on WEHI-164 cells (mouse fibrosarcoma) using the procedure described (see section *Examples,3.2.2.*).

3.2.2 Cytotoxicity assay procedure

Additional reagents required

- Culture medium, *e.g.,* RPMI 1640 containing 10% heat inactivated FCS (fetal calf serum), 2 mM glutamine and 1µg/ml actinomycin C_1 (actinomycin D). If an antibiotic is to be used, additionally supplement media with penicillin/streptomycin or gentamicin*
- Tumor necrosis factor-α, human (hTNF-α) (10 µg/ml)*, sterile*.

Protocol

For the determination of the cytotoxic effect of human tumor necrosis factor-α (hTNF-α) on WEHI-164 cells (mouse fibrosarcoma) (see fig. 5).

Step	Action
1	Preincubate WEHI-164 cells at a concentration of 1 × 10^6 cells/ml in culture medium with 1 µg/ml actinomycin C1 for 3 h at +37°C and 6.5% CO_2.
2	Seed cells at a concentration of 5 × 10^4 cells/well in 100 µl culture medium containing 1 µg/ml actinomycin C_1 and various amounts of hTNF-α (final concentration *e.g.,* 0.001–0.5 ng/ml) into microplates (tissue culture grade, 96 wells, flat bottom).
3	Incubate cell cultures for 24 h at +37°C and 6.5% CO_2 .
4	After the incubation period, add 10 µl of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.
5	Incubate the microplate for 4 h in a humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2).
6	Add 100 µl of the Solubilization solution into each well.
7	Allow the plate to stand overnight in the incubator in a humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2).
8	Check for complete solubilization of the purple formazan crystals and measure the spectrophotometrical absorbance of the samples using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 550 and 600 nm according to the filters available for the ELISA reader, used. The reference wavelength should be more than 650 nm.

3.2.3. Assay procedure for the analysis of neutralizing monoclonal antibodies to growth factors or cytokines

Additional reagents required

- Culture medium, *e.g.,* RPMI 1640 containing heat inactivated 10% FCS (fetal calf serum), 2 mM L-glutamine. If an antibiotic is to be used, additionally supplement media with penicillin/streptomycin or gentamicin*.
- hGM-CSF (10,000 U/ml, 1 µg/ml), sterile *
- anti-hGM-CSF (200 µg/vial), lyophilized, sterile.

Protocol

For the determination of the inhibitory activity of a murine, monoclonal antibody to human granulocyte-macrophage colony stimulating factor (anti-hGM-CSF) on hGM-CSF activity on TF-1 cells (human erythroleukemic cells).

Note: Recombinant, human interleukin-3 (hIL-3)*, which also is effective on TF-1 cells, can be used as a negative control (see fig. 6).

Step	Action
1	Preincubate culture medium containing hGM-CSF (5 U/ml, 0.1 ng/ml) and various amounts of anti-hGM-CSF (final concentration *e.g.,* 0.01–50 µg/ml) in microplates (tissue culture grade, 96 wells, flat bottom).
2	Add TF-1 cells at a concentration of 5 × 10^3 cells/well in 50 µl culture medium and incubate for 48 h.
3	After the incubation period, add 10 µl of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.
4	Incubate the microplate for 4 h in a humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2).
5	Add 100 µl of the Solubilization solution into each well.
6	Allow the plate to stand overnight in the incubator in a humidified atmosphere (*e.g.,* +37°C, 6.5% CO_2).
7	Check for complete solubilization of the purple formazan crystals and measure the spectrophotometrical absorbance of the samples using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 550 and 600 nm according to the filters available for the ELISA reader, used. The reference wavelength should be more than 650 nm.

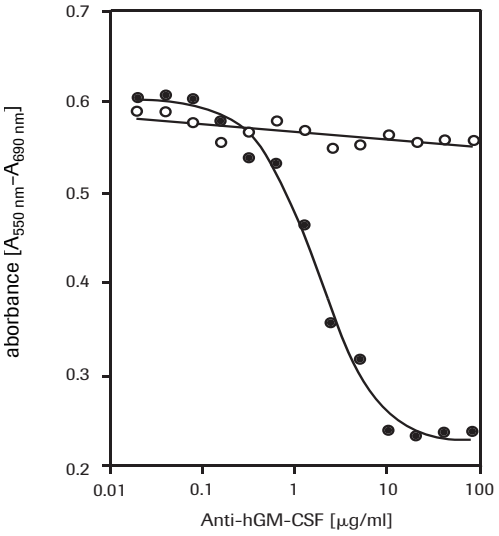

Fig. 6: Inhibition of recombinant human GM-CSF (5 U/ml; 0.1 ng/ml) (●), but not recombinant human interleukin-3 (0.4 U/ml; 0.2 ng/ml) (○) activity on TF-1 cells (human erythroleukemic cells) by anti-hGM-CSF (clone 3092) using the procedure described (see section *Examples, 3.2.3.*).

References

1 Mosmann, T. *et al.* (1983) *J. Immunol. Methods* **65,** 55–63.
2 Tada, H. *et al.* (1986) *J. Immunol. Methods* **93,** 157–165.
3 Denizot, F. & Lang, R. (1986) *J. Immunol. Methods* **89,** 271–277.
4 Gerlier, D. & Thomasset, N. (1986) *J. Immunol. Methods* **94,** 57–63.
5 Hansen, M. B., Nielsen, S. E. & Berg, K. (1989) *J. Immunol. Methods* **119,** 203–210.
6 Vistica, D. T. *et al.* (1991) *Cancer Res.* **51,** 2515–2520.
7 Maehara, Y. *et al.* (1986) *Eur. J. Cancer Clin. Oncol.* **23,** 273–276.
8 Heeg, K. *et al.* (1985) *J. Immunol. Methods* **77,** 237–246.
9 Hooton, J. W. L., Gibbs, C. & Paetkan, U. (1985) *J. Immunol.* **135,** 2464–2473.
10 Mosmann, T. R. & Fong, T. A. T. (1989) *J. Immunol. Methods* **116,** 151–158.
11 Ohno, M. & Abe, T. (1991) *J. Immunol. Methods* **145,** 199–203.
12 Berg, K., Hansen, M. B. & Nielsen, S. E. (1988) *J. Interferon Res.* **8,** (suppl. 1), S. 67.
13 Green, L. M., Reade, J. L. & Ware, C. F. (1984) *J. Immunol. Methods* **70,** 257–268.
14 Espevik, T. & Nissen-Meyer, J. (1986) J. *Immunol. Methods* **95,** 99–105.
15 Ferrari, M., Fornasiero, M. C. & Isetta, A. M. (1990) *J. Immunol. Methods* **131,** 165–172.
16 Van de Loosdrecht, A. A. *et al.* (1991) *J. Immunol. Methods* **141,** 15–22.
17 Cole, S. P. C. (1986) *Cancer Chemother. Pharmacol* **17,** 259–263.
18 Carmichael, J. *et al.* (1987) *Cancer Res.* **47,** 936–942.
19 Twentyman, P. R. & Luscombe, M. (1987) *Br. J. Cancer* **56,** 279–285.
20 Park, J.-G. *et al.* (1987) *Cancer Res.* **47,** 5875–5879.
21 Horiuchi, N. *et al.* (1988) *Cancer Chemother. Pharmacol.* **22,** 246–250.
22 Pieters, R. *et al.* (1988) *Cancer Letters* **41,** 323–332.
23 McHale, A. P. & McHale, L. (1988) *Cancer Letters* **41,** 315–321.
24 Edmondson, J. M*et al.* (1988) *J. Tissue Culture Methods* **11,** 15–17.
25 Faircloth, G. T., Stewart, D. & Clement, J. J. (1988) *J. Tissue Culture Methods* **11,** 201–205.
26 Campling, B. G. *et al.* (1988) *Leukemia Res.* **12,** 823–831.
27 Pieters, P. *et al.* (1989) *Br. J. Cancer* **59,** 217–220.
28 Twentyman, P. R., Fox, N. E. & Rees, K. H. (1989) *Br. J. Haematol.* **71,** 19–24.
29 Plumb, J. A., Milroy, R. & Kaye, S. B. (1989) *Cancer Res.* **49,** 4435–4440.
30 Alley, M. C. *et al.* (1988) *Cancer Res.* **48,** 589–601.
31 Heo, D. S. *et al.* (1990) *Cancer Res.* **50,** 3681–3690.
32 Ruben, R. L. & Neubauer, R. H. (1987) *Cancer Treat. Rep.* **71,** 1141–1149.
33 Rubinstein, L. V. *et al.* (1990) *J. Natl. Canc. Inst.* **82,** 1113–1118.
34 Shanafelt, A. B. (1991) *BioTechniques* **3,** 330.
35 Slater, T. F., Sawyer, B. & Sträuli, U. (1963) Biochim. Biophys Acta **77**, 383-393.
36 Berridge, M. V. & Tan, A. S. (1993) *Arch. Biochem. Biophys.* **303,** 474.

Note: A reference list for microtiter tetrazolium assays (*e.g.,* MTT, XTT, WST-1) is available on request.

Related Products

Parameter	Detection by	Product	Cat. No.
BrdU labeling of proliferating cells	*In situ* assay	▪ BrdU Lab. and Det. Kit I ▪ BrdU Lab. and Det. Kit II ▪ *In Situ* Cell Proliferation Kit, FLUOS	11 296 736 001 11 299 964 001 11 810 740 001
	ELISA	▪ BrdU Lab. and Det. Kit III ▪ Cell Proliferation ELISA, BrdU (colorimetric) ▪ Cell Proliferation ELISA, BrdU (chemiluminescent)	11 444 611 001 11 647 229 001 11 669 915 001
	Single reagents for *in situ* assays and ELISA applications	▪ Anti-BrdU* formalin grade ▪ Anti-BrdU -FLUOS, formalin grade ▪ Anti-BrdU -Peroxidase, Fab fragments, formalin grade	11 170 376 001 11 202 693 001 11 585 860 001
Measurement of metabolic activity	Quantification in microplate	▪ Cell Proliferation Kit I (MTT) ▪ Cell Proliferation Kit II (XTT) ▪ Cell Proliferation Reagent WST-1	11 465 007 001 11 465 015 001 11 644 807 001

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