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Not for use in diagnostic procedures.



# Cell Proliferation Kit I (MTT)

 **Version: 20**

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Colorimetric assay (MTT based) for the nonradioactive quantification of cell proliferation and viability

**Cat. No. 11 465 007 001**    1 kit  
2,500 tests

**Store the kit at  $-15$  to  $-25^{\circ}\text{C}$ .**

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# 1. General Information

## 1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	red	Cell Proliferation Kit I (MTT), MTT labeling reagent, 1x conc.	<ul style="list-style-type: none"> <li>Ready-to-use solution, non-sterile.</li> <li>MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) labeling reagent.</li> <li>5 mg/ml in phosphate-buffered saline (PBS).</li> </ul>	5 bottles, 5 ml each
2	white	Cell Proliferation Kit I (MTT), Solubilization buffer, 1x conc.	<ul style="list-style-type: none"> <li>Ready-to-use solution.</li> <li>10% SDS in 0.01 M HCl.</li> </ul>	3 bottles, 90 ml each

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at  $-15$  to  $-25^{\circ}\text{C}$ , the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	red	MTT labeling reagent, 1x conc.	<p>Store at <math>-15</math> to <math>-25^{\circ}\text{C}</math>.</p> <p><b>⚠ Keep protected from light.</b></p> <p><b>⚠ Repeated freeze-thaw cycles do not affect product stability. If precipitates form during shipment or storage, warm the container to <math>+37^{\circ}\text{C}</math> and mix thoroughly.</b></p> <p>After thawing, store the MTT labeling reagent protected from light at <math>+2</math> to <math>+8^{\circ}\text{C}</math> for up to 4 weeks. Filter reagents through a <math>0.2\ \mu\text{m}</math> pore size membrane before use.</p>
2	white	Solubilization buffer, 1x conc.	<p>Store at <math>-15</math> to <math>-25^{\circ}\text{C}</math>.</p> <p><b>⚠ Repeated freeze-thaw cycles do not affect product stability.</b></p>

### 1.3. Additional Equipment and Reagent required

#### Standard laboratory equipment

- +37°C incubator
- Humidified chamber
- Centrifuge with rotor for microplates (for suspension cells only)
- ELISA reader for microplates.
  - *i* The wavelength to measure the absorbance of the formazan product is between 550 and 600 nm; the reference wavelength should be >650 nm.
- Multichannel pipettes (10 µl, 50 µl, and 100 µl)
- Sterile pipette tips
- Flat-bottomed 96-well microplates, tissue-culture grade

#### For the measurement of the proliferation of 7TD1 cells after IL-6 stimulation

- Culture medium, such as DMEM containing 10% heat inactivated FCS (fetal calf serum), 2 mM L-glutamine, 0.55 mM L-arginine, 0.24 mM L-asparagine-monohydrate, 50 µM 2-mercaptoethanol, 1x HT media supplement, containing 0.1 mM hypoxanthine and 16 µM thymidine
  - *i* If an antibiotic will be used, supplement media with Penicillin-Streptomycin\* or gentamicin.
- Interleukin-6, human (hIL-6)\* (200,000 U/ml, 2 µg/ml), sterile filtered

#### For the measurement of the cytotoxic effect of human tumor necrosis factor-α (hTNF-α) on WEHI-164 cells

- Culture medium, such as RPMI 1640 containing 10% heat inactivated FCS, 2 mM L-glutamine and 1 µg/ml actinomycin C1 (actinomycin D)
  - *i* If an antibiotic will be used, supplement media with Penicillin-Streptomycin\* or gentamicin.
- Tumor Necrosis Factor-α, human (hTNF-α)\* (10 µg/ml), sterile filtered

#### For the analysis of neutralizing monoclonal antibodies to growth factors or cytokines

- Culture medium, such as RPMI 1640 containing 10% heat inactivated FCS, 2 mM L-glutamine
  - *i* If an antibiotic will be used, supplement media with Penicillin-Streptomycin\* or gentamicin.
- hGM-CSF (10,000 U/ml, 1 µg/ml), sterile filtered
- Anti-hGM-CSF (200 µg/vial), lyophilized, sterile filtered

### 1.4. Application

The Cell Proliferation Kit I (MTT) can be used in a variety of applications:

- Designed for the spectrophotometric quantification of cell growth and viability without the use of radioactive isotopes.
- Measurement of cell proliferation in response to growth factors, cytokines, and nutrients (Fig. 1).
- Useful for the measurement of cytotoxicity. Examples include the quantification of tumor necrosis factor-α or -β effects (Fig. 2) or macrophage-induced cell death and the assessment of cytotoxic or growth inhibiting agents, such as inhibitory antibodies (Fig. 3).
- For the replacement of the radioactive [<sup>51</sup>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.
- The MTT assay can also be used to study cell activation.

## 2. How to Use this Product

### 2.1. Before you Begin

#### Safety Information

##### Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

##### Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on [dialog.roche.com](http://dialog.roche.com), or upon request from the local Roche office.

## 2.2. Protocols

### Assay protocol

An overview of the steps is shown in the following table:

Step	Description	Volume/well [ $\mu$ l]	Time [Hours]	Temperature [ $^{\circ}$ C]
1	Perform tissue culture using 96-well, tissue-culture grade, flat-bottom microplates.	100	24 – 96	+37
2	Add MTT labeling reagent and incubate in a humidified atmosphere.	10	4	+37
3	Add Solubilization buffer and incubate in a humidified atmosphere.	100	Overnight	+37
4	Evaluate microplate using an ELISA reader at 550 to 600 nm with a reference wavelength of >650 nm.			

**i** If for the initial incubation of the cells, a larger volume of culture medium is required, increase the amount of MTT labeling reagent correspondingly, for example, 20  $\mu$ l MTT labeling reagent when cells are cultured in 200  $\mu$ l culture medium.

- 1 Grow cells in microplates (tissue-culture grade, 96 wells, flat bottom) in a final volume of 100  $\mu$ l culture medium per well, according to the media needs of the cells, in a humidified atmosphere, such as +37 $^{\circ}$ C, 5 to 6.5% CO<sub>2</sub>.  
– 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, incubate cells for 24 to 96 hours.
- 2 After the incubation period, add 10  $\mu$ l of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.
- 3 Incubate the microplate for 4 hours in a humidified atmosphere, such as +37 $^{\circ}$ C, 5 to 6.5% CO<sub>2</sub>.
- 4 Add 100  $\mu$ l of the Solubilization buffer into each well.

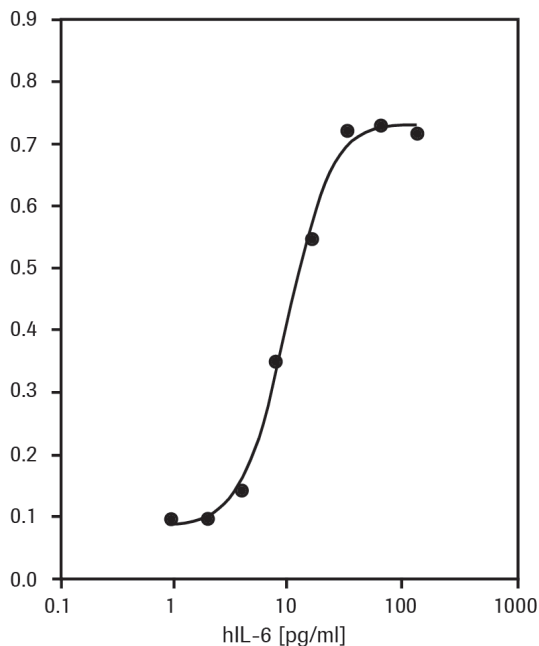
## 2. How to Use this Product

- 5 Allow the plate to stand overnight in the incubator in a humidified atmosphere, such as +37°C, 5 to 6.5% CO<sub>2</sub>.
- 6 Check for total 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. Use a reference wavelength >650 nm.

### Measurement of the proliferation of 7TD1 cells after IL-6 stimulation

For the determination of human Interleukin-6 (hIL-6) activity on 7TD1 cells, see Figure 1.

- 1 Seed 7TD1 cells (mouse-mouse hybridoma) at a concentration of  $2 \times 10^3$  cells/well in 100 µl culture medium containing various amounts of IL-6 (final concentration approximately 0.1 to 10 U/ml [0.001 to 0.1 ng/ml]) into microplates (tissue-culture grade, 96 wells, flat bottom).
- 2 Incubate cell cultures for 4 days at +37°C and 5 to 6.5% CO<sub>2</sub>.
- 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 hours in a humidified atmosphere, such as +37°C, 5 to 6.5% CO<sub>2</sub>.
- 5 Add 100 µl of the Solubilization buffer into each well.
- 6 Allow the plate to stand overnight in the incubator in a humidified atmosphere, such as +37°C, 5 to 6.5% CO<sub>2</sub>.
- 7 Check for total 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. Use a reference wavelength >650 nm.

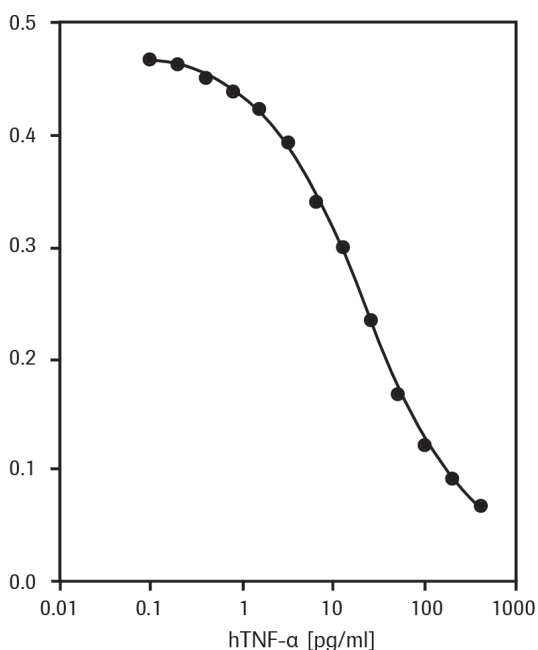


**Fig. 1:** Proliferation of 7TD1 cells (mouse-mouse hybridoma) in response to recombinant human interleukin-6 (hIL-6) using the described protocol.

## Measurement of the cytotoxic effect of human tumor necrosis factor- $\alpha$ (hTNF- $\alpha$ ) on WEHI-164 cells

For the determination of the cytotoxic effect of hTNF- $\alpha$  on WEHI-164 cells, see Figure 2.

- 1 Preincubate WEHI-164 (mouse fibrosarcoma) cells at a concentration of  $1 \times 10^6$  cells/ml in culture medium with 1  $\mu$ g/ml actinomycin C1 for 3 hours at +37°C and 5 to 6.5% CO<sub>2</sub>.
- 2 Seed cells at a concentration of  $5 \times 10^4$  cells/well in 100  $\mu$ l culture medium containing 1  $\mu$ g/ml actinomycin C1 and various amounts of hTNF- $\alpha$  (final concentration approximately 0.001 to 0.5 ng/ml) into microplates (tissue-culture grade, 96 wells, flat bottom).
- 3 Incubate cell cultures for 24 hours at +37°C and 5 to 6.5% CO<sub>2</sub>.
- 4 After the incubation period, add 10  $\mu$ l of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.
- 5 Incubate the microplate for 4 hours in a humidified atmosphere, such as +37°C, 5 to 6.5% CO<sub>2</sub>.
- 6 Add 100  $\mu$ l of the Solubilization buffer into each well.
- 7 Allow the plate to stand overnight in the incubator in a humidified atmosphere, such as +37°C, 5 to 6.5% CO<sub>2</sub>.
- 8 Check for total 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. Use a reference wavelength >650 nm.



**Fig. 2:** Determination of the cytotoxic activity of recombinant human TNF- $\alpha$  on WEHI-164 cells (mouse fibrosarcoma) using the described protocol.

## 2. How to Use this Product

### Analysis of neutralizing monoclonal antibodies to growth factors or cytokines

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).

**i** Recombinant, human interleukin-3 (hIL-3), which is also effective on TF-1 cells, can be used as a negative control (Fig. 3).

- 1 Preincubate culture medium containing hGM-CSF (5 U/ml, 0.1 ng/ml) and various amounts of anti-hGM-CSF (final concentration approximately 0.01 to 50 µg/ml) in microplates (tissue-culture grade, 96 wells, flat bottom).

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- 2 Add TF-1 cells at a concentration of  $5 \times 10^3$  cells/well in 50 µl culture medium and incubate for 48 hours.

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- 3 After the incubation period, add 10 µl of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.

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- 4 Incubate the microplate for 4 hours in a humidified atmosphere, such as +37°C, 5 to 6.5% CO<sub>2</sub>.

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- 5 Add 100 µl of the Solubilization buffer into each well.

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- 6 Allow the plate to stand overnight in the incubator in a humidified atmosphere, such as +37°C, 5 to 6.5% CO<sub>2</sub>.

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- 7 Check for total 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. Use a reference wavelength >650 nm.

## 2.3. Parameters

### Accuracy

The absorbance revealed strongly correlates to the cell number (Fig. 5).

### Sensitivity

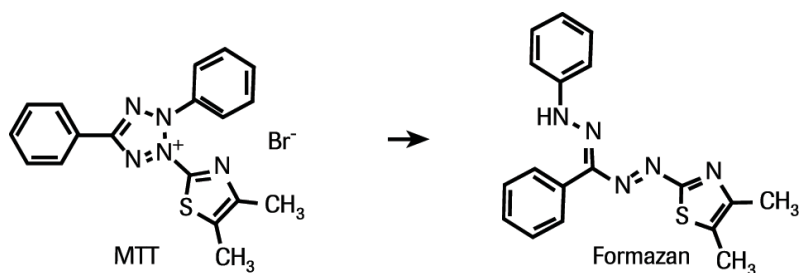
Low cell numbers are detected (Fig. 5).



## 3. Additional Information on this Product

### 3.1. Test Principle

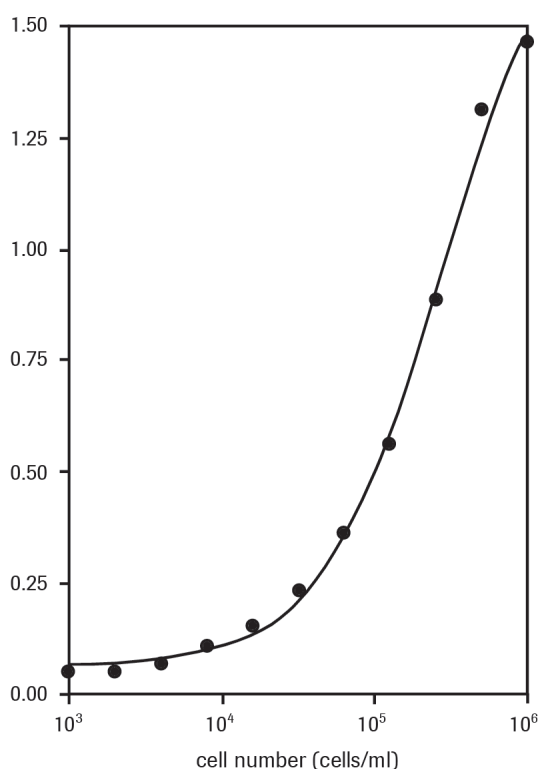
The assay is based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells (Fig. 4). This cellular reduction involves the pyridine nucleotide cofactors NADH and NADPH. The formazan crystals formed are solubilized and the resulting colored solution 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, enables the rapid and convenient handling of a high number of samples.



**Fig. 4:** Metabolization of MTT to a formazan salt by viable cells.

## Overview of the basic steps

- 1 Cells grown in a 96-well tissue-culture plate are incubated with the yellow MTT solution for approximately 4 hours.
- 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 buffer and incubating the plates overnight in a humidified atmosphere, such as +37°C, 5 to 6.5% CO<sub>2</sub>.
- 3 The solubilized formazan product is spectrophotometrically quantified using an ELISA reader.
  - An increase in the 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 (Fig. 5).



**Fig. 5:** Effect of different numbers of cells on color formation (example given using Ag8 cells).

## Background information

The determination of cellular proliferation, viability, and activation are key areas in a wide variety of cellular 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, such as [<sup>3</sup>H] thymidine, or to release a radioisotope, such as [<sup>51</sup>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 immunohistochemistry, in a cell ELISA, and FACS analysis. Cell proliferation and viability assays are of particular importance for routine applications. Tetrazolium salts, such as MTT, XTT, and WST-1 are especially useful for assaying the quantification of viable cells, because they are cleaved to form a formazan dye (Fig. 4; for UV absorbance spectrum, see Figure 6) only by metabolically active cells.

## 4. Supplementary Information

### 4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

#### Text convention and symbols

 *Information Note: Additional information about the current topic or procedure.*

 **Important Note: Information critical to the success of the current procedure or use of the product.**

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

\* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

### 4.2. Changes to previous version

Layout changes.

Editorial changes.

Update to include new safety Information to ensure handling according controlled conditions.

### 4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tumor Necrosis Factor- $\alpha$ , human (hTNF- $\alpha$ )	1,000,000 U, 10 $\mu$ g, 1 ml	11 371 843 001
Interleukin-6, human (hIL-6)	200,000 U, 2 $\mu$ g, 1 ml	11 138 600 001
Penicillin-Streptomycin	for 20 ml, 500x	11 074 440 001

## 4. Supplementary Information

### 4.4. Trademarks

All product names and trademarks are the property of their respective owners.

### 4.5. License Disclaimer

For patent license limitations for individual products please refer to:

**List of biochemical reagent products.**

### 4.6. Regulatory Disclaimer

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

### 4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

### 4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

