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)

1. Kit contents

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

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.

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

Basic steps

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cells, grown in a 96 well tissue culture plate, are incubated with the yellow MTT solution for approx. 4 h.</td>
</tr>
<tr>
<td>2</td>
<td>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, 5 - 6.5% CO2).</td>
</tr>
<tr>
<td>3</td>
<td>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).</td>
</tr>
</tbody>
</table>

Fig. 3: Effect of different numbers of cells on color formation (example given, using Ag8 cells).
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 cytotoxicity (17–34) or growth inhibiting agents such as inhibitory antibodies (see fig. 6).
- For the replacement of the radioactive $^{31}$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.

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

### 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**

Please refer to the following table.

**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, 5 - 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, 5 - 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, 5 - 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.

---

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$^6$ cells/well in 100 μl culture medium containing various amounts of IL-6 (final concentration e.g., 0.1–10 μg/ml) into microplates (tissue culture grade, 96 wells, flat bottom).
2 | Incubate cell cultures for 4 days at +37°C and 5 - 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, 5 - 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, 5 - 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.

---

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$^6$ cells/well in 100 μl culture medium containing various amounts of IL-6 (final concentration e.g., 0.1–10 μg/ml) into microplates (tissue culture grade, 96 wells, flat bottom).
2 | Incubate cell cultures for 4 days at +37°C and 5 - 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, 5 - 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, 5 - 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.

---

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$^6$ cells/well in 100 μl culture medium containing various amounts of IL-6 (final concentration e.g., 0.1–10 μg/ml) into microplates (tissue culture grade, 96 wells, flat bottom).
2 | Incubate cell cultures for 4 days at +37°C and 5 - 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, 5 - 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, 5 - 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.
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 1 mg/ml actinomycin C (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).

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Preincubate WEHI-164 cells at a concentration of 1 × 10⁶ cells/ml in culture medium with 1 μg/ml actinomycin C1 for 3 h at +37°C and 5 - 6.5% CO₂.</td>
</tr>
<tr>
<td>2</td>
<td>Seed cells at a concentration of 5 × 10⁶ cells/well in 100 μl culture medium containing 1 μg/ml actinomycin C, and various amounts of hTNF-α (final concentration e.g., 0.001–0.5 μg/ml) into microplates (tissue culture grade, 96 wells, flat bottom).</td>
</tr>
<tr>
<td>3</td>
<td>Incubate cell cultures for 24 h at +37°C and 5 - 6.5% CO₂.</td>
</tr>
<tr>
<td>4</td>
<td>After the incubation period, add 10 μl of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.</td>
</tr>
<tr>
<td>5</td>
<td>Incubate the microplate for 4 h in a humidified atmosphere (e.g., +37°C, 5 - 6.5% CO₂).</td>
</tr>
<tr>
<td>6</td>
<td>Add 100 μl of the Solubilization solution into each well.</td>
</tr>
<tr>
<td>7</td>
<td>Allow the plate to stand overnight in the incubator in a humidified atmosphere (e.g., +37°C, 5 - 6.5% CO₂).</td>
</tr>
<tr>
<td>8</td>
<td>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.</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Preincubate culture medium containing hGM-CSF (5 U/ml, 0.1 ng/ml) and various amounts of anti-hGM-CSF (final concentration e.g., 0.01–0.5 μg/ml) in microplates (tissue culture grade, 96 wells, flat bottom).</td>
</tr>
<tr>
<td>2</td>
<td>Add TF-1 cells at a concentration of 5 × 10⁴ cells/well in 50 μl culture medium and incubate for 48 h.</td>
</tr>
<tr>
<td>3</td>
<td>After the incubation period, add 10 μl of the MTT labeling reagent (final concentration 0.5 mg/ml) to each well.</td>
</tr>
<tr>
<td>4</td>
<td>Incubate the microplate for 4 h in a humidified atmosphere (e.g., +37°C, 5 - 6.5% CO₂).</td>
</tr>
<tr>
<td>5</td>
<td>Add 100 μl of the Solubilization solution into each well.</td>
</tr>
<tr>
<td>6</td>
<td>Allow the plate to stand overnight in the incubator in a humidified atmosphere (e.g., +37°C, 5 - 6.5% CO₂).</td>
</tr>
<tr>
<td>7</td>
<td>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.</td>
</tr>
</tbody>
</table>
References


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

Related Products

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Detection by</th>
<th>Product</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BrdU labeling of proliferating cells</td>
<td>In situ assay</td>
<td>BrdU Lab. and Det. Kit I</td>
<td>11 296 736 001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BrdU Lab. and Det. Kit II</td>
<td>11 299 964 001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In Situ Cell Proliferation Kit, FLUOS</td>
<td>11 810 740 001</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td>BrdU Lab. and Det. Kit III</td>
<td>11 444 611 001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell Proliferation ELISA, BrdU (colormetric)</td>
<td>11 647 229 001</td>
</tr>
<tr>
<td>Single reagents for in situ assays and ELISA applications</td>
<td></td>
<td>Cell Proliferation ELISA, BrdU (chemiluminescent)</td>
<td>11 669 915 001</td>
</tr>
<tr>
<td>Measurement of metabolite activity</td>
<td>Quantification in microplate</td>
<td>Cell Proliferation Kit I (MTT)</td>
<td>11 465 007 001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell Proliferation Kit II (XTT)</td>
<td>11 465 015 001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell Proliferation Reagent WST-1</td>
<td>11 644 807 001</td>
</tr>
</tbody>
</table>

* available from Roche Diagnostics

Changes to Previous Version

Editorial Changes

Trademarks

Regulatory Disclaimer

Disclaimer of License

For patent license limitations for individual products please refer to: List of biochemical reagent products

Contact and Support

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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany