ab211091

MTT Cell Proliferation Assay Kit

For the measurement of cell proliferation in cultured cells.

View kit datasheet: www.abcam.com/ab211091
(use www.abcam.cn/ab211091 for China, or www.abcam.co.jp/ab211091 for Japan)

This product is for research use only and is not intended for diagnostic use.
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1. Overview

MTT Cell Proliferation Assay Kit (ab211091) provides a simple and accurate method to quantify cell proliferation and viability. The assay is based on the conversion of water soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) compound to an insoluble formazan product. Viable cells with active metabolism convert MTT into formazan. Dead cells, on the other hand, lose this ability and therefore show no signal. Thus color formation serves as a useful and convenient marker of only the viable cells. The measured absorbance at OD 590 nm is proportional to the number of viable cells.

This assay kit provides an easy-to-use, non-radioactive, and high-throughput method for measuring cell proliferation, cell viability and cytotoxicity.

\[ \text{Mitochondrial reductase} \rightarrow \]  
\[ \text{MTT} \quad \text{Formazan} \]

Application:
- Measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients, etc.
- Analysis of cytotoxic and cytostatic compounds such as anticancer drugs, toxic agents and other pharmaceuticals.
- Assessment of physiological mediators and antibodies that inhibit cell growth.
2. Protocol Summary

Grow cells and treat as desired

Discard media and add serum-free media and MTT reagent

Incubate cells at 37°C for 3 hours

Add MTT Solvent

Shake on an orbital shaker for 15 minutes

Measure absorbance at OD590 nm

Determine change as percentage of control after background subtraction
3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.
5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage temperature (before prep)</th>
<th>Storage temperature (after prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTT Reagent</td>
<td>50 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>MTT Solvent</td>
<td>150 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 590 nm
- Pipettes and pipette tips, including multi-channel pipette
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- General cell culture supplies
- Materials and instrumentation necessary to work under sterile conditions
- Foil
8. Technical Hints

- This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.

- Avoid foaming or bubbles when mixing or reconstituting components.

- Avoid cross contamination of samples or reagents by changing tips between sample reagent additions.

- Ensure plates are properly sealed or covered during incubation steps.

- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.

- Make sure all necessary equipment is switched on and set at the appropriate temperature.
9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 MTT Reagent (50 mL):
Ready to use as supplied. Equilibrate to room temperature before use and open vial under sterile conditions. Aliquot reagent so that you have enough volume to perform the desired number of assay. Store at -20°C, protected from light.

9.2 MTT Solvent (150 mL):
Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C. Once opened, use within 2 months.
10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- Culture conditions such as age of the culture, number of passages, and growth media can affect the result and must be taken into consideration when analyzing the data.

**Δ Note** Serum or phenol red present in the culture medium can generate background. If your sample contains serum or phenol red, set up Sample Background Controls.

10.1 Grow cells at varying densities (1-5 x 10^6 cells per mL) in a clear plate according to the desired protocol.

**Δ Note:** Cells seeded at densities between 5,000-10,000 cells/well should reach optimal population densities within 48-72 hours. We recommend using appropriate incubation time depending on the individual cell type and cell concentration used.

10.2 If treating cells, dissolve compounds of interest in an appropriate solvent and treat cells with compound for desired time period.

**Δ Note:** Prepare parallel well(s) as solvent control and use same volume of solvent as for the treated cells.

10.3 Discard treatment media:

10.3.1 For adherent cells, carefully aspirate the media.

10.3.2 For suspension cells, spin the 96 well plate at 1,000 X g, 4°C for 5 minutes in a microplate-compatible centrifuge and carefully aspirate the media.

10.4 Add 50 µL of serum-free media and 50 µL of MTT Reagent into each well.

**Δ Note:** Background control wells: 50 µL MTT Reagent + 50 µL cell culture media (no cells)

10.5 Incubate the plate at 37°C for 3 hours.

10.6 After incubation, remove the MTT Reagent-supplemented media. (Note: when using suspension cells or cells that do not adhere well, spin the 96 well plate at 1,000 X g, 4°C for 5 minutes in a microplate-compatible centrifuge before
removing the supernatant.) Add 150 µL of MTT Solvent into each well.

10.7 Wrap the plate in foil and shake on an orbital shaker for 15 minutes.

10.8 Read absorbance at OD = 590 nm.
11. Data Analysis

CELL PROLIFERATION ASSAYS
- Average the duplicate reading for each sample.
- Subtract the culture medium background from your assay reading. This is the corrected absorbance.
- Amount of absorbance is proportional to cell number.

△ Note: for cell counting, a standard curve can be established with known cell number and fixed incubation times with the assay reagent.

CELL CYTOTOXICITY ASSAYS
- Average the duplicate reading for each sample.
- Subtract the culture medium background from your assay readings. This is the corrected absorbance.
- Calculate percentage cytotoxicity with the following equation, using corrected absorbance:

\[
\% \text{ Cytotoxicity} = \frac{(100 \times (\text{Control} - \text{Sample}))}{\text{Control}}
\]
12. Typical Data

Figure 1. HeLa cells were grown in DMEM media supplemented with 10% FBS, harvested using trypsin and counted using Trypan blue and a hemocytometer. Cells were then serially diluted in a clear cell culture plate and incubated for 3 hours with MTT Reagent at 37°C. After incubation, cells were treated with MTT Solvent for 15 minutes at room temperature. Absorbance was measured at OD = 590 nm. Inset graph is an expanded segment of the assay data at lower cell number per well.
## 13. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Use of ice-cold reagent</td>
<td>Warm reagent to assay temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect</td>
<td>Check equipment and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>wavelength</td>
<td></td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Measured at incorrect</td>
<td>Check equipment and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>wavelength</td>
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</tbody>
</table>
14. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Serum or phenol red present in the cell culture media.