ab197010

MTS Cell Proliferation Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of cell proliferation.

This product is for research use only and is not intended for diagnostic use.
Table of Contents

INTRODUCTION
1. OVERVIEW 2
2. ASSAY SUMMARY 3

GENERAL INFORMATION
3. PRECAUTIONS 4
4. STORAGE AND STABILITY 4
5. MATERIALS SUPPLIED 5
6. MATERIALS REQUIRED, NOT SUPPLIED 5
7. LIMITATIONS 6
8. TECHNICAL HINTS 7

ASSAY PREPARATION
9. REAGENT PREPARATION 8

ASSAY PROCEDURE and DETECTION
10. ASSAY PROCEDURE and DETECTION 9

DATA ANALYSIS
11. TYPICAL DATA 10

RESOURCES
12. TROUBLESHOOTING 11
13. FAQ 12
14. INTERFERENCES 13
15. NOTES 14
1. **OVERVIEW**

MTS Cell Proliferation Assay Kit (Colorimetric) (197010) is a colorimetric sensitive quantification of viable cells in proliferation and cytotoxicity assay. The method is based on the reduction of MTS tetrazolium compound by viable cells to generate a colored formazan product that is soluble in cell culture media. This conversion is thought to be carried out by NAD(P)H-dependent dehydrogenase enzymes in metabolically active cells.

The formazan dye produced by viable cells can be quantified by measuring the absorbance at OD=490-500 nm. The assay can be used for the measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients, etc. It can also be used for the analysis of cytotoxic compounds like anticancer drugs and many other toxic agents and pharmaceutical compounds.

This MTS assay is performed by adding the reagent directly into the cell culture media without the intermittent steps, which are required in the routine MTT assay. In addition, this high-throughput assay requires no washing or solubilization step and can be performed in a 96-well microtiter plate.
2. **ASSAY SUMMARY**

- Culture and treat cells
- Add MTS reagent
- Incubate
- Measure optical density (OD490 nm)
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. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**
Store kit at -20°C in the dark immediately upon receipt. Briefly centrifuge small vials prior to opening. All kit components are supplied as ready to be used. Keep on ice while in use.
Refer to list of materials supplied for storage conditions of individual components.
5. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount (500 tests)</th>
<th>Amount (2500 tests)</th>
<th>Storage Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTS Reagent (in electrocoupling solution)</td>
<td>10 mL</td>
<td>50 mL</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

For frequent use, kit can be stored at 4°C for up to 6 weeks protected from light.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Multi-well spectrophotometer (ELISA reader)
- Colorimetric microplate reader – equipped with filter for OD 490 nm
- 96 well plate: clear, flat bottom plates for colorimetric assay
- Pipettes and pipette tips – preferably a multichannel pipette
- Plate shaker
- SDS
7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- 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.
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.

- Keep enzymes and heat labile components and samples on ice during the assay.

- Make sure all buffers and developing solutions are at room temperature before starting the experiment.

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

- Avoid foaming or bubbles when mixing or reconstituting components.

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

- Make sure you have the appropriate type of plate for the detection method of choice.

- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
9. **REAGENT PREPARATION**

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **MTS Reagent:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.
10. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all controls and samples in duplicate.

10.1 Culture cells (5 – 100 x 10³/well) in a 96-well microtiter plate in a final volume of 200 µL/well. Cells can be cultured in the absence or presence of additional factors for viability/proliferation testing.

If cells are cultured in different volume of culture medium, adjust the amount of MTS Reagent accordingly.

10.2 Incubate cells for 20 – 48 hours. The appropriate incubation time will depend on the individual cell type and cell concentrations used. Therefore, it is recommended to determine the optimal incubation time for a particular experiment.

10.3 Add 20 µL/well MTS Reagent into each well and incubate for 0.5 – 4 hours at 37°C in standard culture conditions.

10.4 Shake the plate briefly on a shaker and measure absorbance of treated and untreated cells using a plate reader at OD=490 nm.

**NOTE:** To measure the absorbance at a later time, add 10 µL of 10% SDS to each well to stop the reaction. Store SDS-treated plates protected from light in a humidified chamber at room temperature for up to 18 hours.
11. **TYPICAL DATA**

Data provided for *demonstration purposes only*.

**Figure 1:** Absorbance at OD=490 nm increases proportionally to cell density. Different amounts of Jurkat cells were cultured overnight at 37°C. in a final volume of 200 µL/well. MTS reagent was added and absorbance at OD=490 nm was recorded using ELISA plate reader. Each point represents a mean of 3 replicates. Assay was performed according to the kit protocol.
## 12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay not working</strong></td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Use of a different 96-well plate</td>
<td>Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate</td>
</tr>
<tr>
<td><strong>Sample with erratic readings</strong></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer, increase number of strokes</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple free/thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>Check protocol for interfering substances; deproteinize samples</td>
</tr>
<tr>
<td><strong>Lower/Higher readings in samples and Standards</strong></td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Allowing reagents to sit for extended times on ice</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td><strong>Unanticipated results</strong></td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/below the linear range</td>
<td>Concentrate/Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
13. FAQ
14. INTERFERENCES
15. NOTES
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