Ab211091 – MTT Cell Proliferation Assay Kit

For the measurement of cell proliferation in cultured cells.

For overview, typical data and additional information please visit: www.abcam.com/ab211091 (use www.abcam.cn/ab211091 for China, or www.abcam.co.jp/ab211091for Japan)

This product is for research use only and is not intended for diagnostic use

Precautions

- 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.

Storage and Stability: Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 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.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
MTT Reagent	50 mL	-20°C	-20°C
MTT Solvent	150 mL	-20°C	-20°C

Materials Required, Not Supplied

These materials are not included, 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

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.

Reagent Preparation: Briefly centrifuge small vials at low speed prior to opening.

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 no. of assay.
   Store at -20°C, protected from light.
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.
   Tips for dissolving MTT crystals - add warm solvent (37°C max). Do not add cold MTT solvent.

Assay Procedure

- Equilibrate all materials and prepared reagents to RT just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.
- 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.

ΔNOTE: 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

1. Grow cells at varying densities (1-5 x106 cells per mL) in a clear plate according to the desired protocol.

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.

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

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

3. Discard treatment media:
4. For adherent cells, carefully aspirate the media.
5. 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.
6. Add 50 µL of serum-free media and 50 µL of MTT Reagent into each well.

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

7. Incubate the plate at 37°C for 3 hours.
8. After incubation, add 150 µL of MTT Solvent into each well.
9. Wrap the plate in foil and shake on an orbital shaker for 15 minutes.
10. Read absorbance at OD = 590 nm.

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.
  ΔNOTE: 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:

$$\%\ Cytotoxicity = \frac{(100\ x\ (Control - Sample))}{Control}$$

Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold reagent	Warm reagent to assay temperature
	Plate read at incorrect wavelength	Check equipment and filter settings of instrument
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter settings of instrument

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.

FAQs

Should the media in background control well be serum-free media? Also, after 3-hour incubation with MTT reagent, should I remove MTT reagent or directly add MTT solvent without removing MTT reagent?
Use serum-free media for the background control wells too. Whether to remove the MTT reagent or not depends on the type of cells you have. For adherent cells, you can remove the MTT reagent gently and then add the MTT solvent. For suspension cells, one may lose the cells while removing the MTT reagent. Thus, we do not recommend removing the MTT reagent. The MTT solvent can be added directly and the total volume will be around 250 µl in the wells.

What are the known interreferences with this assay?
A variety of chemical compounds are known to interfere with the MTT assay. These are mostly reducing compounds that lead to non-enzymatic reduction of the MTT to formazan. Examples include ascorbic acid, vitamin A, sulfhydryl-containing compounds including reduced glutathione, coenzyme A, and dithiothreitol. Certain plant extracts and polyphenolic compounds can also interfere with the MTT assay.

Can you use media containing phenol red with this assay?
It's preferred not to use phenol red.

Why do I see high absorbance from media only?
The media contaminated with cells/bacteria or it might contain ascorbic acid. Always use sterile technique to work with the reagents. Try to work with sterile pipette tips and inside a biological hood.

Why is the MTT reagent blue/green in colour?
The reagent is either contaminated by a reducing agent or cell/bacterial contamination or it received excessive exposure to light. The reagent should be discarded. New reagent should be processed under sterile conditions and kept in the dark at 4°C.

Does MTT-medium need to be removed before addition of solvent?

Whether to remove the MTT reagent or not depends on the type of cells you have. For adherent cells, you can remove the MTT reagent gently and then add the MTT solvent. For suspension cells, for the fear that you may lose cells, please do not remove MTT reagent and you can add the MTT solvent directly and the total volume will be around 250 µl in the well.

Technical Support

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