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ab232856
XTT assay kit

For the measurement of cell proliferation in adherent and non-adherent cells.

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

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1. Overview

XTT cell proliferation assay kit provides an easy to use tool for studying induction and inhibition of cell proliferation in any *in vitro* model. The assay is based on the extracellular reduction of XTT by NADH produced in the mitochondria via trans-plasma membrane electron transport and an electron mediator. Reduction of XTT produces a water-soluble formazan which dissolves directly into the culture medium, eliminating the need for an additional solubilization step. This kit will allow investigators to screen drug candidates involved in cell cycle regulation.

Culture cells in a 96-well plate in a CO_2 incubator at 37°C for 24-48 hours.

Add 10 µl XTT Mixture to each well and incubate for 2 hours (adherent cells) to 4 hours (non-adherent cells) in a CO_2 incubator at 37°C

Measure absorbance at 450 nm

2. Materials Supplied and Storage

Store kit at -20°C immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for several months at -20°C.

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

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
XTT Developer Reagent	600 µl	-20°C	-20°C
Electron Mediator Solution	600 µl	-20°C	-20°C

3. 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 450 nm
- 96 well plate

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

- Briefly centrifuge small vials at low speed prior to opening.
- Thaw all components to room temperature (18-25°C) prior to use.

1. Combine equal volumes of XTT Developer Reagent and Electron Mediator Solution to make enough XTT Mixture for the number of wells in your experiment (10 µl/well) and mix well.
2. If the entire volume will not be used in a single experiment, we recommend that you aliquot and store it at -20°C. When stored at -20°C the XTT Mixture will be stable for several months. Avoid repeated freeze/thaw cycles.

6. Sample Preparation

Seed cells in a 96-well plate at a density of 10^3-10^5 cells/well in 100 µl of culture medium with or without compounds to be tested. Culture the cells in a CO2 incubator at 37°C for 24-48 hours.

7. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

1. Add 10 µl of the prepared XTT Mixture to each well using a repeating pipettor.
2. Mix gently for one minute on an orbital shaker.
3. Incubate the cells for two hours (adherent cells) to four hours (non-adherent cells) at 37°C in a CO_2 incubator.
4. Before reading the plate mix gently on an orbital shaker for one minute to ensure homogenous distribution of color.
5. Measure the absorbance of each sample using a microplate reader at 450 nm.

8. FAQs / Troubleshooting

General troubleshooting points can be found at www.abcam.com/assaykitguidelines.

9. Typical Data

Data provided **for demonstration purposes only.**

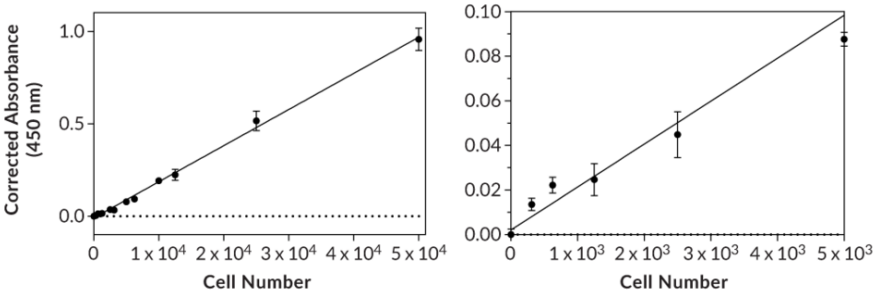

Figure 1. A typical cell titration experiment using HL-60 target cells

10. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)