Version 2 Last updated 11 May 2023

ab273300
Acid Phosphatase Assay Kit (Cytotoxicity)

View Kit datasheet: https://www.abcam.com/ab273300
(use https://www.abcam.cn/ab273300 for china, or
https://www.abcam.co.jp/ab273300 for Japan)

For quantification of cell viability / cytotoxicity via measurement of lysosomal Acid Phosphatase activity.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

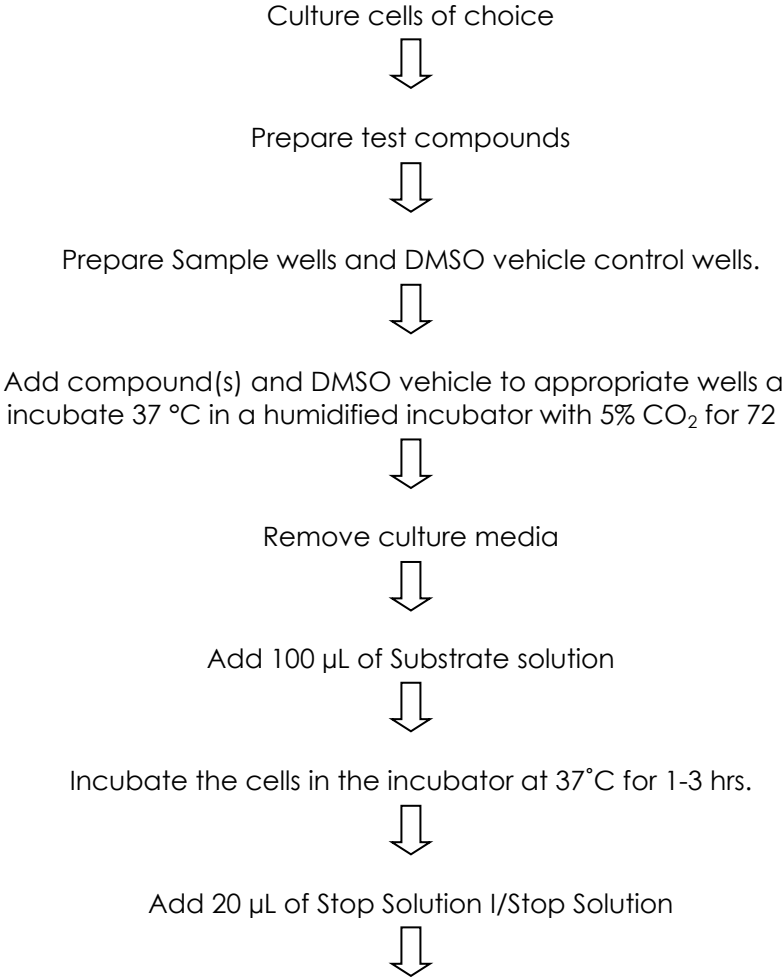
Table of Contents

1. Overview

In the Acid Phosphatase Assay Kit (ab273300), intracellular lysosomal Acid Phosphatase (ACP) cleaves the colorless chromogenic substrate para-nitrophenyl phosphate producing a yellow colored product (p-nitrophenol) under alkaline conditions. The released chromophore can be measured at OD 405 nm and is directly proportional to the viable cell number.

This assay kit is simple, accurate, reproducible and sensitive with a dynamic range between 10^3 -10^5 cells. It offers a simple, yet excellent and efficient method for in vitro cytotoxicity studies as well as high-throughput drug screening.

2. Protocol Summary



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 4°C in the dark immediately upon receipt. Kit has a storage time of 12 months 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

Item	Quantity	Storage temperature
ACP Buffer	50 mL	4°C
pNPP Substrate/Substrate (20 tabs)(Lyophilized)	2 vials	4°C
Stop Solution I/Stop Solution	10 mL	4°C
Doxorubicin (20mM)	100 µL	4°C

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 405 nm
- Cell line of choice
- 96-well clear plate with flat bottom

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, standard and 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.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- 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 **ACP Buffer:**
Supplied ready to use. Bring to room temperature before use.

9.2 **Stop Solution I/Stop Solution:**
Supplied ready to use. Bring to room temperature before use.

9.3 **Doxorubicin (20mM):**
Supplied ready to use. Bring to room temperature before use.

9.4 **pNPP Substrate/Substrate:**
For 100 assays (1 plate), dissolve 4 tablets of pNPP Substrate/Substrate in 10 mL of ACP Buffer to prepare Substrate solution. Prepare solution prior to your experiments. Use substrate solution within 24 hours.
Discard unused Substrate solution. Protect from light.

10. Sample Preparation

10.1 Cell Culture

10.1.1 Grow cells to ~80% confluency.

10.2 Adherent cells:

10.2.1 Trypsinize and spin down cells.
10.2.2 Add 5 mL of culture media to disperse the cells.
10.2.3 Determine the cell density using a hemocytometer.
10.2.4 Adjust the cell concentration if necessary.
10.2.5 Add 100 µL of the cell suspension (typically containing between 5,000–20,000 cells/well) into a 96-well clear flat-bottom plate.
10.2.6 Incubate overnight under sterile conditions at 37 °C and 5% CO_2.

10.3 Non-adherent cells

10.3.1 Grow the cells to ~80% confluency.
10.3.2 Spin down the cells.
10.3.3 Add culture media to adjust cell concentration.
10.3.4 Add 100 µL of the cell suspension typically containing between 5,000–20,000 cells/well to a 96-well clear flat bottom plate.
10.3.5 Incubate overnight under sterile conditions at 37 °C and 5% CO_2.

11. Assay Procedure

11.1 Compound Treatment:

11.1.1 Prepare stock solution of the compounds of interest using DMSO as solvent.
11.1.2 Dilute compound stock solution in DMSO appropriately.

Δ Note: Recommended maximum final DMSO concentration in the assay should be 1%.

11.1.3 Add compounds to the wells.
11.1.4 Prepare a DMSO vehicle control and a background control containing only the medium.
11.1.5 For inhibitor control, add 1 µL of 20 mM doxorubicin to a well containing cells.
11.1.6 Incubate the plate at 37 °C in a humidified incubator with 5% CO_2 for 72 hr.
11.1.7 Concurrently incubate a control culture without treatment.

11.2 Adherent cells

11.2.1 Remove the culture media.

11.3 Non-adherent cells

11.4 Spin down cells and remove the culture media carefully without disturbing the cells.

11.5 Assay Protocol:

11.6 Add 100 µL of the Substrate solution into each well.
11.7 Incubate the cells in the incubator at 37˚C for 1-3 hours depending on cell density

Δ Note: Lower density may need longer incubation times.

11.8 After incubation, add 20 µL of Stop Solution I/Stop Solution into each well.
11.9 Measure the O.D. at 405 nm.

12. Calculations

12.1.1 Correct the background by subtracting the O.D. of the background control from all readings.

12.1.2 Calculate the percentage of inhibition using the formula below:

$$\%\ Inhibition\ = \frac{OD_{vehicle}\ -\ OD_{sample}}{OD_{vehicle}} \text{ x } 100\%$$

$OD_{vehicle}$ = solvent control (e.g. DMSO) after background correction.

OD_{sample} = sample after background correction.

13. Typical Data

Dose-response curve – data provided **for demonstration purposes only**. A new dose-response curve must be generated for each assay performed.

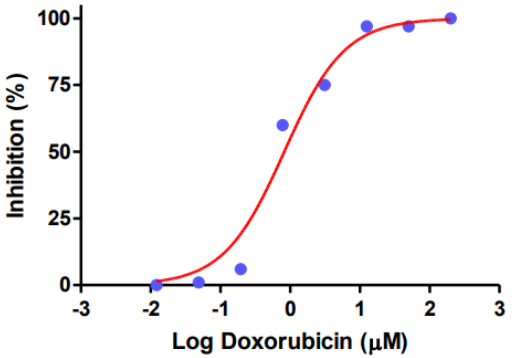


Figure 1. Dose-response curve of MCF7 cells exposec to doxorubicin for 72 hr as determined by the ACP cytotoxicity assay. Assays were performed according to the kit protocol in triplicate. The determined IC_{50} is 0.82 µM.

14. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

15. 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)