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Acid Phosphatase Cell Cytotoxicity Assay Kit

(Catalog # K937-500; 500 assays, Store kit at 4°C)

I. Introduction:

The measurement of the lysosomal Acid Phosphatase (ACP) enzymatic activity has become one of the common methods used to detect and quantify cell viability or drug cytotoxicity due to ubiquitous of this enzymatic activity among all organisms. Under normal conditions, eukaryotic cells exhibit abundant cell-membrane associated ACP activity; however ACP activity is considerably lower when cells are exposed to cytotoxic agents. As a result, measurement of the ACP activity in cells is a reliable method to quantify cell growth in both adherent and non-adherent cells. Compared with other cell proliferatioin assays based on the reduction of tetrazolium salts, ACP cytotoxicity assay displays higher sensitivity and reproducibility. In this cell-based assay, intracellular 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. BioVision's ACP cytotoxicity assay kit is simple, accurate, reproducible and sensitive with a dynamic range between 10^3-10^5 cells. This kit offers a simple, yet excellent and efficient method for *in vitro* cytotoxicity studies as well as high-throughput drug screening.

II. Application:

- *In vitro* cell proliferation cytotoxicity studies
- High-throughput drug screening

III. Sample Type:

- Cell culture: Adherent and suspension cells

IV. Kit Contents:

Components	K937-500	Cap Code	Part Number
ACP Buffer	50 ml	NM	K937-500-1
Substrate (20 tabs)	2 vials	Blue	K937-500-2
Stop Solution	10 ml	WM	K937-500-3
20 mM Doxorubicin	100 µl	Red	K937-500-4

V. User Supplied Reagents and Equipment:

- 96-well clear flat-bottom plate
- Multi-well spectrophotometer
- Personal Protective equipment: gloves, goggles and laboratory coat

VI. Storage Conditions and Reagent Preparation:

Store kit at 4°C. The kit components are stable for one year when stored as recommended. Read the entire protocol before performing the experiment.

- **ACP Buffer, Stop Solution and Doxorubicin**: Bring to room temperature before use.
- **Substrate**: For 100 assays (1 plate), dissolve 4 tablets of 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.*

VII. ACP Cytotoxicity Assay:

1. **Cell Culture**: Grow cells to ~80% confluency. **Adherent cells:** trypsinize and spin down cells. Add 5 ml of culture media to disperse the cells. Determine the cell density by using a hemocytometer. Adjust the cell concentration if necessary. Add 100 µl of the cell suspension (typically containing between 5,000–20,000 cells/well) into a 96-well clear flat-bottom plate. Incubate overnight under sterile conditions at 37 °C and 5% CO_2. **Non-adherent cells**: grow the cells to ~80% confluency. Spin down the cells and add culture media to adjust cell concentration. Add 100 µl of the cell suspension typically containing between 5,000–20,000 cells/well to a 96-well clear flat-bottom plate. Incubate overnight under sterile conditions at 37 °C and 5% CO_2.
2. **Compound Treatment:** Prepare stock solution of the compounds of interest using DMSO as solvent. Dilute compound stock solution in DMSO appropriately. Recommended maximum final DMSO concentration in the assay should be 1%. Add compounds to the wells. Prepare a DMSO vehicle control and a background control containing only the medium. For inhibitor control, add 1 µl of 20 mM doxorubicin to a well containing cells. Incubate the plate at 37 °C in a humidified incubator with 5% CO_2 for 72 hr.
3. **ACP Assay**: **Adherent cells**: remove the culture media. **Non-adherent cells**: spin down cells and remove the culture media carefully without disturbing the cells. Add 100 µl of the Substrate solution into each well and incubate the cells in the incubator at 37°C for 1-3 hrs. depending on cell density (lower density may need longer incubation times). After incubation, add 20 µl of Stop Solution into each well.
4. **Measurement:** Measure the O.D. at 405 nm.
5. **Calculations:** Correct the background by subtracting the O.D. of the background control from all readings. Calculate the percentage of inhibition using the formula below:

$$\% \text{ Inhibition} = \frac{\text{O.D.}_{\text{vehicle}} - \text{O.D.}_{\text{sample}}}{\text{O.D.}_{\text{vehicle}}} \times 100\%$$

Where: $\text{O.D.}_{\text{vehicle}}$ is the O.D. of the solvent control (eg. DMSO) after background correction

O.D.$_{sample}$ is the O.D. of the sample after background correction.

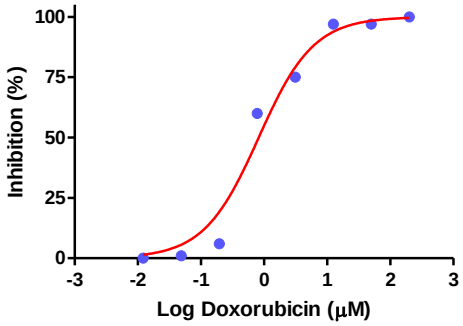

Figure: Dose-response curve of MCF-7 cells to doxorubicin for 72 hr determined by the ACP cytotoxicity assay. Assays were performed according to the kit protocol in triplicate. The determined IC_{50} is 0.82 µM.

VIII. RELATED PRODUCTS:

Neutral Red Cytotoxicity Assay Kit (K447)
Crystal Violet Cell Cytotoxicity Assay Kit (K329)
LDH-Cytotoxicity Colorimetric Assay Kit (K311)
Bioluminescence Cytotoxicity Assay Kit (K312)
PicoProbe™ LDH-Cytotoxicity Fluorometric Assay Kit (K314)
MTT Cell Proliferation Assay Kit (Colorimetric) (K299)
MTS Cell Proliferation Colorimetric Assay Kit (K301)
Sulforhodamine B Cell Cytotoxicity Assay Kit (K943)
WST-NR-CV Combined Cytotoxicity Assay Kit (K543)
LDH-Cytotoxicity Colorimetric Assay Kit II (K313)
Senescence Detection Kit (K320)
PicoProbe™ Lactate Dehydrogenase Activity Assay Kit (K730)
ADP Colorimetric/Fluorometric Assay Kit (K355)
ATP Colorimetric Assay Kit II (K354)

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