Crystal Violet Cell Cytotoxicity Assay Kit

(Catalog # K329-1000; 1000 assays, Store kit at -20°C)

I. Introduction:

Crystal violet (CV) cell cytotoxicity assay is one of the common methods used to detect cell viability or drug cytotoxicity. CV is a triarylmethane dye that can bind to ribose type molecules such as DNA in nuclei. Normally, dead adherent cells will detach from cell culture plates and will be removed from viable cell population during washing steps. CV staining can be used to quantify the total DNA of the remaining population and thus determine cell viability. The CV staining is directly proportional to the cell biomass and can be measured at 570 nm. CV staining is a quick and versatile assay for screening cell viability under diverse stimulation or inhibition conditions. BioVision's CV cell cytotoxicity assay kit is simple, accurate, reproducible and sensitive. It also includes Doxorubicin as a positive control. This kit offers an excellent and efficient method for *in vitro* cytotoxicity studies as well as high-throughput drug screening.

II. Application:

- *In vitro* cell proliferation and cell cytotoxicity studies
- High-throughput drug screening of potential cytotoxic compounds

III. Sample Type:

- Cell culture: Adherent cells

IV. Kit Contents:

Components	K329-1000	Cap Code	Part Number
Crystal Violet Staining Solution	40 ml	WM	K329-1000-1
10X Washing Solution	115 ml	NM	K329-1000-2
Solubilization Solution	100 ml	WM	K329-1000-3
20 mM Doxorubicin	100 µl	Red	K329-1000-4

V. User Supplied Reagents and Equipment:

- 96-well clear flat-bottom plate
- Multi-well spectrophotometer
- Personal Protective equipment: gloves, goggles and laboratory coat
- 100% Methanol

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment. Bring all reagents to room temperature before use.

- **Crystal Violet Staining Solution**: Bring it to room temperature before use. **Add 11 ml of 100% methanol (not supplied) into the bottle**. Shake contents and let it stand for 15 minutes at room temperature. After use, store it at -20°C.
- **10X Washing Solution**: Add 1 part of 10X Washing Solution to 9 parts deionized water to make 1X Washing Solution. Store at 4°C.
- **Solubilization Solution**: Bring it to room temperature before use. After use, store it at room temperature.
- **20 mM Doxorubicin:** Thaw doxorubicin before use. Store at -20°C.

VII. Crystal Violet Cell Cytotoxicity Assay:

1. **Cell Culture**: Grow adherent cells to ~80% confluency. Trypsinize and spin down the cells. Add 5 ml of growth medium to disperse the cells. Determine the cell density by using a hemocytometer. Adjust the cell concentration if necessary. Add 200 µl of the cell suspension (25,000-100000 cell/ml) to a 96-well clear flat-bottom plate in order to seed 5000-20000 cells/well. Let the cells settle down overnight and adhere to the plate.
2. **Compound Treatment:** Prepare compounds using DMSO as solvent. Dilute compound stock solution in DMSO appropriately. Recommended final DMSO concentration in wells should be 0.5% or less. Add compounds to the wells. Prepare a DMSO vehicle control and a background control (cell culture growth media). For inhibitor control: add 1 µl of 20 mM doxorubicin to a well containing the cells. Incubate the plate at 37 °C in a humidified incubator with 5% CO_2 for 72 hr.
3. **CV Staining**: Remove the culture medium. Wash cells with 200 µl of 1X Washing Solution. *Washing should be done as gentle as possible to avoid disturbance of the cell monolayer*. *Remove wash solutions as much as possible by pipetting*. Add 50 µl of Crystal Violet Staining Solution (with Methanol) to each well and stain for 20 min at RT. After incubation, remove the staining solution. Use 200 µl of 1X Washing Solution to wash the cells. Wash the cells for 4 times. At the end of the 4th washing step, remove washing solutions as much as possible by pipetting and air-dry the plate if necessary.
4. **Solubilization:** Add 100 µl of Solubilization Solution to each well. Shake the plate occasionally or place the plate on a shaker for 20 min at room temperature.
5. **Measurement:** Measure the O.D. at 595 nm.
6. **Calculations:** Correct the background by subtracting the O.D. of the background control from all readings. Calculate the percentage of cytotoxicity using the formula below:

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

Where: $O.D._{DMSO}$ is the O.D. of the DMSO control after background correction
$O.D._{sample}$ is the O.D. of the sample after background correction.

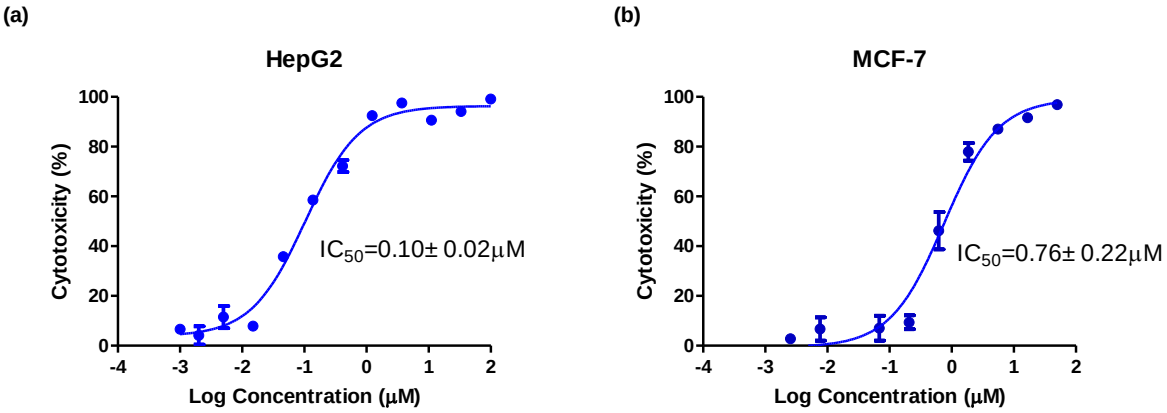

Figure: (a) Dose-response curve of HepG2 (a) and MCF-7 (b) cells to doxorubicin for 72 hr determined by the CV staining assay. Assays were performed according to the kit protocol in triplicate.

VIII. RELATED PRODUCTS:

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)
BrdU Cell Proliferation Assay Kit (K306)
StayBrite™ Highly Stable ATP Bioluminescence Assay kit (K791)
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)
ApoSENSOR™ ADP/ATP Ratio Bioluminescence Assay Kit (K255)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)

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