CPT-0102

Crystal Violet Cell Cytotoxicity Assay

(1000 wells, Store at -20°C)

Introduction:

Crystal violet binding as a measure of cytotoxicity is a well established method for detection of cell viability or drug cytotoxicity. Crystal violet is a triarylmethane dye that binds to ribose containing molecules such as DNA. Typically, dead cells detach from cell culture plates and are removed during washing steps. Using crystal violet to quantify the DNA of the remaining population allows for a quick measure of cell viability by monitoring its absorbance at 570 nm. Use primarily for adherent cultures, crystal violet staining is directly proportional to biomass useful for screening cell viability under a wide range of conditions. **AkrivisBio's Crystal Violet Cell Cytotoxicity Assay** is simple, sensitive means to study cell proliferation, viability or cytotoxicity.

Kit Contents:

Crystal Violet Stain	40 ml	NM	CPT-0102A
Wash Solution Concentrate	115 ml	NM	CPT-0102B
Solubilization Solution	100 ml	WM	CPT-0102C
Doxorubicin	100 µl	Red	CPT-0102D

User Supplied Materials:

100% Methanol

Storage and Handling:

Store the unopened kit at -20°C. Centrifuge all small vials briefly, prior to opening.
Crystal Violet Stain: Warm to room temperature before use. Dilute with 11 ml of 100% methanol. Close bottle, shake and let stand for 15 min. Store at -20°C.
Wash Solution Concentrate: Add 1 part of Wash Solution Concentrate to 9 parts DI water to make Wash Solution. Store at 4°C.
Solubilization Solution: Bring to room temperature before use. Can be stored at room temperature or with other components.
Doxorubicin (20 mM): Bring to room temperature before use. Store at -20°C.

Assay Protocol:

1. Cell Culture: Grow adherent cells to ~80% confluency. Trypsinize and centrifuge the cells at 500 X g. Add 5 ml of growth medium to disperse the cells. Determine the cell density using a hemocytometer. Adjust the cell concentration to 25,000 – 100,000/ml, if necessary. Add 200 µl of the cell suspension to a 96-well clear flat-bottom plate to seed 5000-20000 cells/well. Allow the cells to settle overnight and adhere to the plate.

2. Treatment: Prepare challenge compounds using DMSO as solvent. Keep the compound concentration high enough to keep DMSO below 0.5% in the cell culture. Add compounds to the wells. Prepare a DMSO vehicle control and a background control containing just growth media). As inhibitor control add 1 µl of 20 mM doxorubicin to a well containing cells. Incubate the plate under cell culture conditions (37°C, humidified incubator, 5% $CO_{2)}$ for 72 hr.

3. Crystal Violet Staining: Remove the culture medium. Wash cells with 200 µl of Wash Solution. *Washing should be done as gently as possible.* Remove as much wash solution as possible. Add 50 µl of Crystal Violet Stain methanol solution to each well and wait 20 min at room temperature. After incubation, remove methanol stain solution. Wash with 200 µl Wash Solution, 4 times. After the 4th wash step, remove wash solution as much as possible and air-dry the plate.

4. Solubilization: Add 100 µl of Solubilization Solution to each well. Shake the plate for 20 min at room temperature.

5. Measurement: Measure the OD at 570 nm.

6. Calculations: Subtract the background control reading from all readings. Calculate the percentage of cytotoxicity using the formula below:

$$\% \text{ Cytotoxicity} = \frac{OD_{DMSO} - OD_{SAMPLE}}{OD_{DMSO}} \times 100\%$$

Where: OD_{DMSO} is the OD of the DMSO control after background correction
OD_{Sample} is the OD of the sample after background correction.

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

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