Version 22-04/24

User's Manual

Crystal violet Assay Kit (Cell viability)

REF **DEIA-NS2309-1**

Σ **1000T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For the measurement of cell viability or drug cytotoxicity in adherent cells.

General Description

Crystal violet Assay Kit (Cell viability) offers an excellent and efficient method for in vitro cytotoxicity studies as well as highthroughput drug screening. It is simple, accurate, reproducible and sensitive. It includes Doxorubicin as a positive control. The Crystal violet staining is directly proportional to the cell biomass and can be measured at 570 nm. This type of staining is a quick and versatile assay for screening cell viability under diverse stimulation or inhibition conditions.

Reagents And Materials Provided

1. Crystal Violet Staining Solution, 40 mL, before prep store at -20°C, after prep store at -20°C
2. 10× Washing Solution, 115 mL, before prep store at -20°C, after prep store at 4°C
3. Solubilization Solution, 100 mL, before prep store at -20°C, after prep store at RT
4. 20 mM Doxorubicin, 100 µL, before prep store at -20°C, after prep store at -20°C

Materials Required But Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

1. Microplate reader capable of measuring absorbance at OD570 nm.
2. 96 well plate with clear flat bottom.
3. 100% methanol.

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.

Aliquot components in working volumes before storing at the recommended temperature. Avoid repeated freeze-thaws of reagents.

Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Crystal violet Staining Solution

1. Bring to room temperature.
2. Add 11 ml of 100% methanol (not supplied) into the bottle.

3. Shake contents and let it stand for 15 minutes at room temperature.
4. After use store at -20°C.

10× Washing Solution

1. Add 1 part of 10X Washing Solution to 9 parts deionized water to make 1X Washing Solution.
2. After use store at 4°C.

Solubilization Solution

1. Bring to room temperature.
2. After use store at RT.

20 mM Doxorubicin

1. Thaw Doxorubicin before use.
2. After use store at -20°C.

Assay Procedure

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

Assay all standards, controls and samples in duplicate.

Cell Culture:

1. Grow adherent cells to ~80% confluency.
2. Trypsinize and spin down the cells.
3. Add 5 mL of growth medium to disperse the cells.
4. Determine the cell density by using a hemocytometer.
5. Adjust the cell concentration if necessary.
6. Add 200 µL of the cell suspension (25,000-100000 cell/mL) to a 96-well clear flat-bottom plate to seed 5000-20000 cells/well.
7. Let the cells settle down overnight and adhere to the plate.

Compound Treatment:

1. Prepare compounds using DMSO as solvent.
2. Dilute compound stock solution in DMSO appropriately.

Δ Note Recommended final DMSO concentration in wells should be 0.5% or less.

3. Add compounds to the wells.
4. Prepare a DMSO vehicle control and a background control (cell culture growth media).
5. For inhibitor control: add 1 µL of 20 mM Doxorubicin to a well containing the cells
6. Incubate the plate at 37°C in a humidified incubator with 5% CO_2 for 72 hours.

Crystal violet Staining:

1. Remove the culture medium.
2. Wash cells with 200 µL of 1X Washing Solution.

Δ Note Washing should be done as gentle as possible to avoid disturbance of the cell monolayer.

3. Remove wash solutions as much as possible by pipetting.
4. Add 50 µL of Crystal Violet Staining Solution (with methanol) to each well and stain for 20 minutes at RT.
5. After incubation, remove the staining solution.
6. Use 200 µL of 1× Washing Solution to wash the cells.
7. Wash the cells four times.
8. At the end of the washing step, remove washing solutions as much as possible by pipetting and air-dry the plate if necessary.

Solubilization:

1. Add 100 µL of Solubilization Solution to each well.
2. Shake the plate occasionally or place the plate on a shaker for 20 minutes at room temperature.

Measurement:

1. Measure the O.D. at 570 nm.

Calculation

1. Correct the background by subtracting the O.D. of the background control from all readings.
2. Calculate the percentage of cytotoxicity using the formula below:

$$\%\ Cytotoxicity = \frac{OD\ DMSO - OD\ sample}{OD\ DMSO}\ x\ 100\%$$

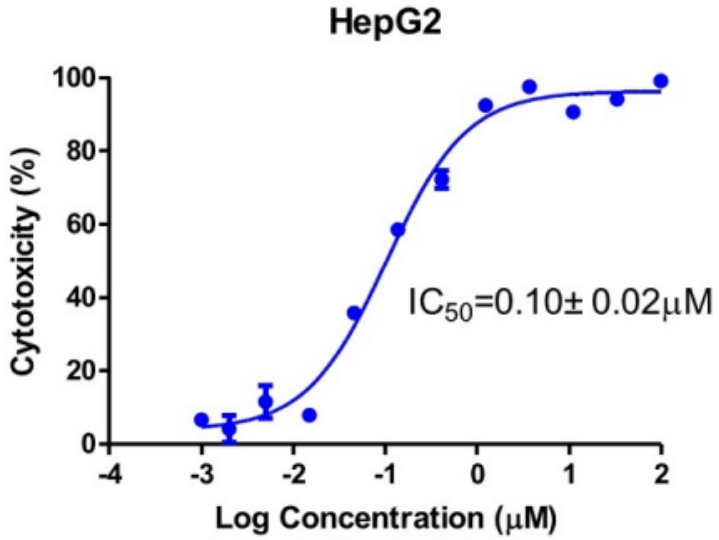
Where:

OD DMSO is the OD of the DMSO control after background correction.

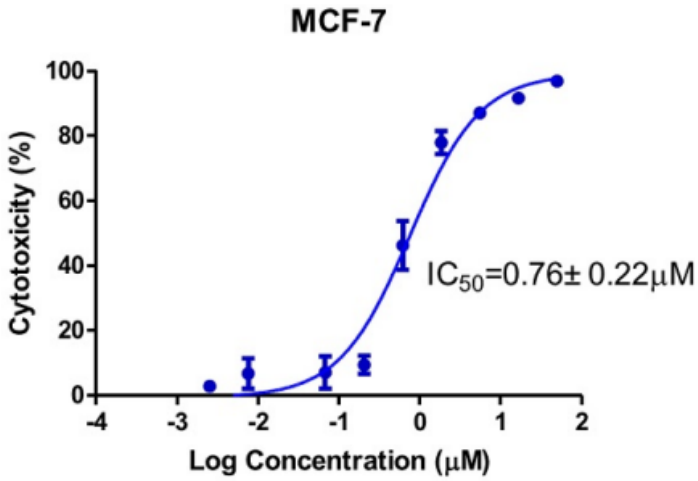
OD sample is the OD of the sample after background correction.

Typical Standard Curve

Data provided for demonstration purposes only



Dose-response curve of HepG2 (Human liver hepatocellular carcinoma cell line) cells to Doxorubicin for 72 hours determined by the Crystal violet Assay Kit (Cell viability). Assays were performed according to the kit protocol in triplicate.



Dose-response curve of MCF7 (Human breast adenocarcinoma cell line) cells to Doxorubicin for 72 hours determined by the Crystal violet Assay Kit (Cell viability). Assays were performed according to the kit protocol in triplicate.

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