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PERFORMING A CRYSTAL VIOLET ASSAY FOR LIVING CELL MASS NORMALIZATION FOLLOWING A CYRIS® EXPERIMENT

Abstract

The following crystal violet (CV) assay can be used to determine living cell mass as an indicator of cytotoxicity or cell proliferation of adherent cell cultures after performance of a CYRIS® experiment.

The assay is based on CV staining of living cells attached to the cell culture plate. Adherent cells that have undergone cell death, detach from the cell culture plate and are washed away during the assay. The remaining living cells are stained with CV, which is a cationic triphenylmethane dye that can be used to stain nucleic acids such as DNA. After several washing steps with PBS, CV is released from the stained cells by adding trisodium citrate buffer and can be measured by absorbance at 550 nm.

The quantity of CV is directly proportional to the living cell mass attached to the plate. The living cell mass can be used to deduce the level of cytotoxicity or cell proliferation.

INTRODUCTION

Crystal violet (CV) is a cationic triphenylmethane dye (Figure 1) that can be used for Gram stain in order to differentiate between Gram-negative or Gram-positive bacteria. As a DNA stain it can also be applied to determine cell viability or cell death in different in vitro assays. The CV assay is a simple and widely used method for in vitro cytotoxicity studies. It is believed that adherent cells detach from the culture plate when they undergo cell death. In the CV staining based assay, only living cells attached to the cell culture plate are stained with CV. Dead cells are washed away during the assay. The CV staining is directly proportional to the living cell mass and can be measured at 550 nm.

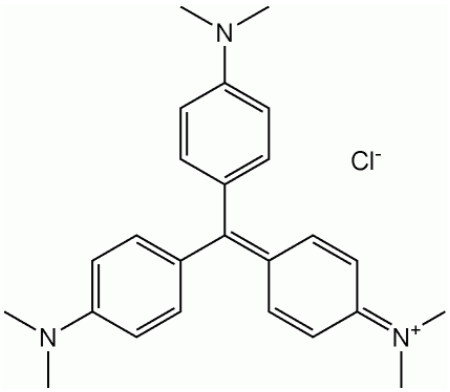

Figure 1. Chemical structure of crystal violet – a cationic triphenylmethane dye.

ASSAY WORKFLOW

Perform A Cyris® Experiment

Stain The Cells With Crystal Violet

Solubilize The Crystal Violet From CV-Stained Cells And Measure The Absorbance

MATERIALS REQUIRED

Reagents

- Crystal violet
- 100% methanol
- Milli-Q water
- Trisodium citrate
- 50% ethanol
- Sodium chloride (NaCl)
- Potassium chloride (KCl)
- Disodium hydrogen phosphate (Na_2HPO_4)
- Kaliumdihydrogenphosphat (KH_2PO_4)
- Magnesium chloride ($MgCl_2$)
- Calcium chloride ($CaCl_2$)

Devices and disposable materials

- Incyton Cyris® flox
- Incyton metabolic sensor plate
- Microplate reader capable of measuring absorbance at 550 nm
- 96-well-plate with clear flat bottom
- Vacuum pump, flexible tube and glass pasteur pipettes
- Pipettes and pipette tips (2-200µl, 50-1000µl)

GENERAL PRECAUTIONS

We recommend to carefully read all safety instructions of the manufacturer before using CV, methanol and ethanol. Wear protective gloves, protective clothing and eye protection when working with CV, methanol and ethanol. Avoid the release of CV to the environment.

REAGENT PREPARATION

PBS with calcium and magnesium (pH 7.4)

Reagent	Amount
NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.15 g
KH_2PO_4	0.2 g
$MgCl_2$	0.024 g
$CaCl_2$	0.055 g
Adjust pH to 7.4	
Milli-Q water	add to 1 l

0.5% crystal violet solution

Reagent	Amount
Crystal violet	1.25 g
100% methanol	50 ml
Milli-Q water	add to 250 ml

Trisodium citrate buffer

Reagent	Amount
Trisodium citrate	7.35 g
Milli-Q water	dissolve in 250 ml
50% ethanol	add 250 ml

CV STAINING PROCEDURE

- After the Cyris experiment is completed, take the sensor plate out of the instrument, remove the lid of the plate, and aspirate the medium via the side channels using a glass pipette connected to a vacuum pump
- Wash the wells once with 500 µl PBS with calcium and magnesium
- Do not pipette directly onto the cells but rather to the wall of the wells
- Pipette carefully to avoid detaching the cells
- Aspirate the PBS via the side channels
- Add 150 µl of the crystal violet solution per well and incubate at RT for 10 min
- Aspirate the crystal violet solution via the side channels
- Wash carefully two to three times with 300 µl Milli-Q water per well and aspirate the water via the side channels (wash until no crystal violet is left)
- Let the plate dry for at least overnight (Figure 2)

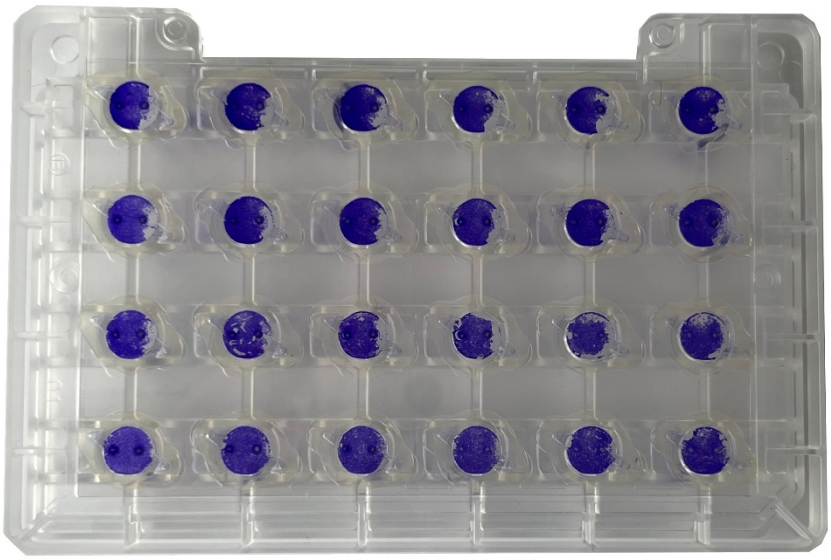

Figure 2. Cells attached in every well of the sensor plate stained with CV and dried overnight.

SOLUBILIZATION OF CV AND MEASURMENT

- Add 200 µl trisodium citrate buffer to solubilize the CV and place the plate on a shaker slightly shaking for 10 min at RT
- Transfer 100 µl of the solubilized CV into a 96-well-plate with clear flat bottom and seal the plate with the lid
- Avoid touching the bottom of the 96-well-plate
- Transfer the plate without lid into a microplate reader and measuring the absorbance at 550 nm
- OD value should be between 0.1-1.3, otherwise dilute your samples

NORMALIZATION PROCEDURE

- To normalize your OCR, ECAR, and Impedance divide every measured raw value by the CV OD value of the corresponding well
- Afterwards you can form groups with similar treatment and make statistical evaluations

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