



Crystal violet staining for identification of cell growth issues



Crystal violet staining is a simple and rapid method to observe unusual and non-uniform patterns in cell proliferation. Staining makes it easier to detect growth problems in adherent cells.

The following protocol describes the staining of adherent cells with crystal violet.

Documentation is required when questioning or reporting unusual cell growth. Images of the entire growth area, as well as a detail section, are desirable.

Note

Adhere to national regulations when handling biological material and use appropriate protective clothing.

Warning:

Crystal violet is harmful if inhaled, swallowed, or absorbed through the skin. Contact can cause cancer and severe eye irritation [1].

Please observe the necessary protective measures when handling hazardous substances. For detailed information, refer to the manufacturer's safety data sheets.

Material:

- Cells cultivated under sterile conditions in culture vessels
- D-PBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$
- Anhydrous methanol ($\geq 99,8\%$)
- Crystal violet solution 0.1 % (w/v)
- D-PBS: methanol, 1 :1
- Tap water
- Deionized water
- Filter paper
- Container for hazardous liquid waste

Method

Note: This protocol is described for a 96-well plate format. It can be applied to other plate sizes by scaling cell numbers and reagent volumes accordingly.

- Aspirate and discard the cell culture medium
- Wash the cells carefully:
 - Add D-PBS (e.g., 0.1–0.2 mL per well of a 96-well plate)
 - Remove and discard the wash solution
- Pre-treatment:
 - Add D-PBS: methanol (1:1) (0.2 mL/cm²)
 - Incubate for 2 minutes at room temperature
 - Remove the solution
- Fixation:
 - Add pure methanol (0.2 mL/cm²)
 - Incubate for 10 minutes at room temperature
 - Remove methanol completely
- Allow the plate to drain and air dry briefly



- Staining:
 - Add crystal violet solution (0.1% w/v) (0.2 mL/cm²)
 - Ensure the cell layer is completely covered
 - Incubate for 10 minutes at room temperature
 - Discard staining solution into hazardous waste
- Washing steps:
 - Gently wash 2–3 times with tap water
 - Follow with 2–3 washes using deionized water
 - Important:
 - Do not direct the water stream onto the cell layer
 - Hold the plate at an angle
 - Fill well and immediately aspirate
- Remove residual liquid:
 - Invert the plate onto paper towels and tap gently
- Allow cells to air dry completely at room temperature
- Analysis:
 - Examine stained cells under a microscope
 - Document results

Literature:

^[1] Sigma Aldrich Crystal violet solution V5265

Amanda Capes-Davis, R. Ian Freshney (2010) Freshney's Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications (8th Ed.) 429 -430

Feoktistova M, Geserick P, Leverkus M. Crystal Violet Assay for Determining Viability of Cultured Cells. Cold Spring Harb Protoc. 2016 Apr 1;2016(4)

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