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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 (gentian violet).

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

Note

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

Observe the rules of aseptic working during all steps.

Warning:

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

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

Material:

D-PBS
Methanol
Crystal violet 0.1% (w/v)
Adherent cell line
Cell culture medium
Water
Deionized water

Pipettes
Pipetting aid

Method

- Aspirate and discard the medium from the cell culture.
- Rinse the cell monolayer with an appropriate volume of D-PBS (e.g., 1-2 mL) to loosen dead cells, then discard the rinse.
- Fix the cells with 100% methanol (at room temperature) and incubate for 10 minutes.
- Aspirate and discard the methanol.
- Add the crystal violet solution (prepare or use a 0.1% solution) and incubate for 10 minutes at room temperature.
- Aspirate and discard the staining solution.
- Wash the cell monolayer 2-3 times with tap water, followed by 2-3 washes with deionized water to remove unbound dye.
- Allow the cells to air dry at room temperature.
- The stained cell monolayer can be examined under a microscope, and the results can be documented.

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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