



## Crystal Violet Staining for Identification of Cell Growth Issues

Crystal violet staining is a simple and rapid method for observing unusual and non-uniform patterns of cell proliferation. Staining makes it easier to detect growth problems in adherent cells. The following protocol describes crystal violet (gentian violet) staining of adherent cells. 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:

Follow the national regulations for handling biological materials and wear the appropriate protective clothing.  
During the work process, be sure to observe the rules of aseptic technique.

### Warning:

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

Please observe the necessary precautions when handling hazardous substances. For detailed information, 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

1. Aspirate and discard the fluid.
2. Rinse the cell monolayer with D-PBS to detach dead cells and discard the rinse.
3. Fix the cells with 100 % methanol and incubate for 10 minutes.
4. Aspirate and discard the methanol.
5. Add the crystal violet solution and incubate for 10 minutes at room temperature.
6. Aspirate and discard the staining solution.
7. Wash the unbound dye with tap water and then with deionized water.
8. After washing, allow the cells to dry at room temperature.
9. Examine the stained cell monolayer and document the result.

### Literature:

[1] Sigma Aldrich Crystal violet solution V5265

Freshney, I. R. Culture of animal cells: a manual of basic technique and specialized application – 7ed. Wiley Blackwell 2016: 370 -371

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