Hoechst 33342 Nuclear Staining Protocol

Overview

Hoechst 33342 is a cell-permeant nuclear stain that binds to A-T rich regions of DNA. It is widely used in fluorescence microscopy, flow cytometry, and cell imaging. This protocol provides guidance for staining both live and fixed cells using aqueous Hoechst 33342 solutions.

Staining Live Cells

1. Prepare Hoechst 33342 working solution (0.1-10 µg/ml) in culture medium or PBS.
2. Add dye directly to cells and incubate at 37°C for 10-30 minutes.
3. Rinse cells gently with PBS (optional, depending on background).
4. Image cells using appropriate filters (excitation ~350 nm, emission ~461 nm).

Staining Fixed Cells

1. Fix cells using paraformaldehyde or methanol-based fixation.
2. Rinse cells with PBS.
3. Prepare working solution of Hoechst 33342 (e.g., 1 µg/ml in PBS).
4. Incubate fixed cells with the dye for 10 minutes at room temperature.
5. Wash with PBS and proceed to imaging.

Formulation Notes

- The 20 mM (11.2 mg/ml) solution offers greater flexibility for dilution and minimizes added volume.
- The 5 mg/ml solution may be preferable for protocols requiring a lower stock concentration or larger pipetting volumes.
- Both formulations are supplied in water and can be used for live or fixed cell imaging.

General Notes

- Protect Hoechst 33342 from light to prevent photodegradation.
- Optimize concentration based on cell type and staining requirements.
- Thorough washing may reduce background in flow cytometry applications.