DAPI Nuclear Staining Protocol

Overview

DAPI (4',6-diamidino-2-phenylindole) is a fluorescent stain that binds strongly to A-T rich regions of DNA. It is commonly used to visualize nuclei in both live and fixed cells in fluorescence microscopy.

Staining Live Cells

1. Prepare a working solution of DAPI (e.g., 0.1-1 µg/ml) in PBS or culture medium.
2. Add DAPI directly to the live cell culture.
3. Incubate at 37°C for 10-15 minutes.
4. Wash gently with PBS to reduce background.
5. Image immediately using a DAPI-compatible filter (excitation ~358 nm, emission ~461 nm).

Staining Fixed Cells

1. Fix cells using paraformaldehyde (e.g., 4% in PBS) or methanol.
2. Wash with PBS.
3. Prepare a DAPI working solution (e.g., 0.1-1 µg/ml in PBS).
4. Incubate fixed cells with DAPI for 5-10 minutes at room temperature.
5. Rinse with PBS and proceed to fluorescence imaging.

Formulation Notes

- The aqueous 1 mg/ml formulation is ready-to-use or can be diluted for working solutions.
- The powder form offers maximum shelf-life and customizable stock concentrations.
- Both formats yield consistent nuclear staining results in fixed or live cell workflows.

General Notes

- DAPI is photostable but should be protected from prolonged light exposure.
- Optimize concentration and incubation time depending on cell type and imaging conditions.
- Store solutions protected from light at 2-8°C or -20°C for long-term storage.