Staining Live Cells with Fluorescent Nucleic Acid Stain LUCS 13

LUCS 13 is a cell-permeant nucleic acid stain that exhibits green fluorescence upon binding to nucleic acids. The stain has a high fluorescent yield and a structure identical to SYTO™13 stain.

LUCS 13 is used to stain both DNA and RNA in live and dead eukaryotic cells as well as Gram-positive and Gram-negative bacteria. The dye is excited by the blue laser at 488 nm. Its fluorescence emission is detected in the fluorescein channel with a peak at 509 nm when bound to DNA and 514 nm when bound to RNA.

The dye can be used in simultaneous labeling with cell-impermeant nuclear markers, such as YoDi-3, to evaluate cell viability using fluorescence microscopy and flow cytometry.

Protocol

The exact protocol depends on the specific cell type and experimental task. In general, it can be described as follows:

1. Prepare a 50 µM stock solution of LUCS 13. To do this, add 990 µl of deionized water to 10 µl of a 5 mM LUCS 13 solution. The stock solution can be aliquoted and frozen for long-term storage.
2. To prepare a working 1 µM LUCS 13 solution, add 20 µl of 50 µM LUCS13 stock to 980 µl of PBS.
3. Carefully remove adherent cells from the growth surface in a suitable manner. With suspension cells, start work from the next point.
4. Wash the cells once with cold PBS (pH 7.4). Seed cells by centrifugation for 5 min at 300 *g*.
5. Incubate the cells in 1 ml of 1 µM LUCS 13 working solution for 30 min at 37°C.
6. Remove the dye solution by centrifugation for 5 min at 300 *g*.
7. Wash the cells once with PBS.
8. *(Optional)* If necessary, cells can be fixed in 3.7% formaldehyde in PBS for 15 min, washed with PBS, and restained with another stain.

Important

- Use plastic vials when handling LUCS 13 solutions, as the diluted dye may stick to the glass.
- The brighter staining results can be obtained with phosphate-free buffers (e.g.,15 mM HEPES in saline).
- Before starting the experiment, test several dye concentrations ranging from 10 nM to 5 µM to determine the most optimal dye dilution. The result of staining is influenced by many factors: growth medium, cell density, presence of other cell types, duration of staining, etc.