Lumiprobe
Life science solutions

Staining Cells with Fluorescent Nucleic Acid Stain LUCS® 5

LUCS® 5 (1,5-bis{[2-(di-methylamino) ethyl]amino}-4,8-dihydroxyanthracene-9,10-dione or DRAQ5®) is a cell-permeant far-red fluorescent DNA dye for live and fixed cell imaging and analysis.

LUCS® 5 readily permeates the cell membranes and intercalates between A-T bases of double-strained DNA (dsDNA). The dye has a high affinity to dsDNA and shows negligible binding to RNA and mtDNA. LUCS® 5 does not exhibit a fluorescence enhancement on DNA binding; thus, the measured fluorescence is proportional and stoichiometric to the quantity of nuclear DNA content of the cell.

Due to its cell permeability, LUCS® 5 is useful for assessing DNA content and cell cycle but is not suitable for use as a viability dye. As with other cell-permeant DNA intercalating dyes, LUCS® 5 may inhibit cell division in long-term assays, so this effect should be tested before the experiment.

In microscopy and high-content screening, LUCS® 5 is useful as a nuclear counterstain for both live and fixed specimens. In flow cytometry, LUCS® 5 allows direct blood and bone marrow cell discrimination without preliminary red blood cell lysis, fixation, permeabilization, or RNase treatment.

LUCS® 5 has absorbance peaks at 603 nm and 646 nm and an emission peak at 697 nm (when intercalated into dsDNA). Thus, this dye is spectrally compatible with the most common labels, such as GFP, FITC, R-PE, or RFP. LUCS® 5 also has high photostability and demonstrates no photobleaching effect during imaging.

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Before you start

- LUCS® 5 solution should be stored at 2–8 °C. Do NOT freeze the solution! LUCS® 5 may precipitate out of the solution when frozen. Re-dissolving it may not be easy.
- Sodium azide interferes with LUCS® 5 staining; thus, it is recommended to stain in Dulbecco's Phosphate Buffered Saline (DPBS), PBS (without sodium azide), or culture medium.
- The commonly used dye concentration to stain cells is 5–20 µM. The working dilution depends on the cell type and density and should be defined experimentally.
- The optimal cell density and staining duration for DNA content analysis may vary by cell type. The staining protocol should be optimized in preliminary experiments for best results.
- Note that dsDNA-bound dye will fluoresce brightly in the nucleus, and the unbound dye may fluoresce dimly in the cytoplasm, allowing segmentation of the cytoplasmic and nuclear compartments.

Cell Staining

Staining of Live Cells for Nuclei Visualization

1. Grow cells on a sterile coverslip. Adherent cells can be stained directly on the coverslip.
2. Dilute LUCS® 5 solution to 5–20 µM in complete medium or other azide-free buffer immediately before use.
3. Add LUCS® 5 solution to samples and incubate at 37 °C for 5–30 minutes in the dark.
4. Aspirate LUCS® 5 staining solution and wash cells twice with 1× PBS.

   (Optional) Cells may also be analyzed without washing, but this may increase the background from unbound dye.
5. For fluorescent microscopy, mount cells under a coverslip using a mounting medium.
6. Proceed to imaging. We recommend using a 715LP or longer wavelength filter, though the dye is well-detected in filters typically used to detect AF 647 (e.g., 660/20 or 692/40 nm).

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Staining of Fixed Cells for Nuclei Visualization

1. Grow cells on a sterile coverslip. Adherent cells can be stained directly on the coverslip.
2. Fix and permeabilize cells as desired.
3. Dilute LUCS® 5 solution to 5–20 µM in 1× PBS or other azide-free buffer immediately before use.
4. Add LUCS® 5 solution to samples and incubate for 5–30 minutes at room temperature in the dark.
5. Rinse samples once in 1× PBS.

   (Optional) Cells may also be analyzed without washing, but this may increase the background from unbound dye.
6. For fluorescent microscopy, mount cells under a coverslip using a mounting medium.
7. Proceed to imaging. We recommend using a 715LP or longer wavelength filter, though the dye is well-detected in filters typically used to detect AF 647 (e.g., 660/20 or 692/40 nm).

Staining of Live Cells for DNA Content Analysis by Flow Cytometry

1. Obtain a single-cell suspension.
2. Resuspend cells at a density of 0.5×10^6 cells/mL or less in a complete medium or other azide-free buffer containing 20 µM LUCS® 5.
3. Incubate at 37 °C for 5–15 minutes in the dark.
4. Pellet cells by centrifugation at 400 *g* for 3–4 minutes at room temperature and aspirate LUCS® 5 staining solution.
5. Resuspend cells in 1× DPBS and immediately proceed to analysis by flow cytometry. Cells may also be analyzed without washing, but this may decrease DNA content histogram resolution.
6. Analyze cells on a cytometer equipped with the 633 nm red laser. If cell cycle analysis is performed, detection in the AF 700 channel with a 680LP or 715LP filter might help with the resolution of the emission peaks.

   (Optionally) On a flow cytometer, it is possible to detect LUCS® 5 using 488 nm blue laser excitation.

Staining of Fixed Cells for DNA Content Analysis by Flow Cytometry

1. Obtain a single-cell suspension.
2. Fix cells on ice for 30 minutes with 70–80% ice-cold ethanol.
3. Wash cells once with 1× DPBS.
4. Dilute LUCS® 5 solution to 20 µM in 1× DPBS or other azide-free buffer immediately before use.
5. Stain cells at a density of 0.5×10^6 cells/mL or less for 5–15 minutes at room temperature in the dark.
6. No further wash is necessary prior to analysis.
7. Analyze cells on a cytometer equipped with the 633 nm red laser. If cell cycle analysis is performed, detection in the AF 700 channel with a 680LP or 715LP filter might help with the resolution of the emission peaks.

   (Optionally) On a flow cytometer, it is possible to detect LUCS® 5 using 488 nm blue laser excitation.

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Spectral properties:

Excitation:

- Maxima at 603 nm and 646 nm;
- 647 nm line optimal;
- 488, 514, 568 and 633 nm lines, sub-optimal;
- Two-photon excitation at 1047 nm.

Emission (instrument dependent):

- Maxima at 681 nm (free) / 701 nm (intercalated with dsDNA);
- Detection from 665 nm to near-infrared (NIR);
- Emission filters may include 695L, 715LP, or 780 LP.

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