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Life science solutions

Staining Cells with Lucifer Yellow CH Dye

Lucifer Yellow CH is a highly sensitive fluorescent dye widely used in neurobiology, cytology, and molecular biology for cell tracking, studying intercellular interactions, and visualizing neurons.

Lucifer Yellow CH is suitable for labeling both live and fixed preparations using microinjection, iontophoresis, and electroporation methods[1]. Due to the presence of a carbohydrazide group (CH) in its composition, the dye is retained in cells after fixation with aldehydes (e. g., paraformaldehyde or glutaraldehyde), making it compatible with most immunohistochemistry and cytochemistry protocols.

Lucifer Yellow CH has a high quantum yield and is resistant to photobleaching, making it ideal for confocal and fluorescence microscopy. The dye also possesses photoconversion properties and can be used to visualize labeled cellular structures by bright-field and electron microscopy[2].

Before you begin

- Since Lucifer Yellow CH has two negative charges, it does not penetrate the intact membranes of living cells; therefore, the primary methods of introducing the dye into cells are microinjection, iontophoresis, and electroporation.
- Although Lucifer Yellow CH is a hydrophilic compound, some cells can absorb it by endocytosis and can be labeled in this way.
- Lucifer Yellow CH is not suitable for long-term tracking in living cells due to the gradual washout of the dye.
- For simultaneous labeling with other fluorophores (e. g., DAPI), ensure there is no spectral overlap.

Working solution preparation

1. Dissolve Lucifer Yellow CH in distilled water, 0.5–1 M LiCl, or culture medium to a concentration of 0.2–5%. The choice of solvent and dye concentration depends on the loading method used. The Lucifer Yellow CH concentrations given can serve as starting points; the exact dye concentration should be determined experimentally.
2. Filter the resulting solution through a 0.22 µm filter to remove undissolved particles.
3. Store the solution at 4 °C in a light-protected place for no more than 1 month.
4. Unused solution can also be stored at -20 °C or -80 °C in a light-protected place. Avoid repeated freezing and thawing.

Microinjection loading

1. Fill the microinjection needle with a 1–5% solution of Lucifer Yellow CH in bidistilled water. The exact dye concentration should be determined empirically.
2. Place the cells or tissue section on the stage of a fluorescence microscope equipped with a micromanipulator.
3. Inject the dye into the target cell under microscope control.

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4. Incubate for 10–15 minutes at room temperature.
5. After injection, wash the sample to remove excess unincorporated dye.
6. Visualize the stained cells using a fluorescence microscope with the appropriate filter set (excitation maximum at ~430 nm, emission maximum at ~540 nm).

Iontophoresis loading

1. Prepare a working 1–2% solution of Lucifer Yellow CH dye in 0.5–1 M LiCl. This solvent is preferred for iontophoretic injection because it increases the dye's mobility. The exact dye concentration should be determined empirically.
2. Fill a glass microelectrode with the working solution of Lucifer Yellow CH.
3. Place the cells or tissue section on the stage of a fluorescence microscope equipped with a micromanipulator.
4. Use the micromanipulator to insert the microelectrode into the desired cell under visual control.
5. Apply a negative current pulse (e. g., 0.2–0.5 nA for 5–15 minutes) to load the negatively charged Lucifer Yellow CH into the cell.
6. After injection, rinse the sample to remove excess unincorporated dye.
7. Visualize the stained cells using a fluorescence microscope with the appropriate filter set (excitation maximum at ~430 nm, emission maximum at ~540 nm).

Electroporation loading

1. Prepare a 0.2–0.5% working solution of Lucifer Yellow CH in calcium-free culture medium.
2. Rinse the cells with fresh calcium-free culture medium, avoiding drying them out.
3. Transfer the cells to the electroporation chamber.
4. Add 400 µL of the working solution of Lucifer Yellow CH to the cells.
5. Apply an electrical pulse using an electroporation device according to the manufacturer's instructions.
6. Immediately replace the dye solution with pre-warmed calcium-free medium containing 10% dialyzed serum. This will help close the pores formed during electroporation.
7. Incubate the cells for 3–5 minutes in an incubator to allow the pores to close and the dye to transfer through the gap junctions.
8. Wash the cells with fresh medium to remove unincorporated dye.
9. Visualize the stained cells using a fluorescence microscope with the appropriate filter set (excitation maximum at ~430 nm, emission maximum at ~540 nm).

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Photoconversion

Lucifer Yellow CH allows visualization of labeled cellular structures using light and electron microscopy methods, thanks to the formation of a stable, optically and electron-dense precipitate after photooxidation with intense blue light in the presence of a diaminobenzidine (DAB) solution[2].

1. Incubate the sample in blocking buffer (50 mM glycine, 5 mM KCN , and 5 mM aminotriazole) for 15 minutes to reduce non-specific background reaction of diaminobenzidine derivatives. Wash thoroughly after incubation.
2. Add a filtered 0.1–0.2% DAB solution to the sample and incubate for 10–20 minutes at room temperature.
3. Place the sample under a microscope equipped with a light source. Illuminate the target area with intense blue light (e. g., a 150 W xenon lamp). Use an FITC filter set to excite Lucifer Yellow CH.

   (*Optional*) A constant flow of pure oxygen through the solution during photooxidation significantly enhances the reaction.
4. Stop illuminating the sample when a light brown staining appears in the labeled cells, indicating the formation of a visible precipitate (usually about 10 minutes).
5. Wash the cells with buffer to remove unbound reagents.
6. Visualize the stained cells using light or electron microscopy.

Fixation and permeabilization

1. For light, fluorescence, and confocal microscopy, fix the stained samples with 4% paraformaldehyde for 10–20 minutes at room temperature or 4 °C.
2. Wash the cells with PBS, then permeabilize with 0.1–0.5% Triton X-100 solution in PBS for 5–10 minutes. After this, the samples can be stained using standard immunocytochemistry and immunohistochemistry protocols.
3. For detection of Lucifer Yellow CH by electron microscopy, fix the samples sequentially with 2.5% glutaraldehyde and 1% OsO_4 in 0.1M cacodylate buffer (pH 7.5) for 1 and 3 hours, respectively. After this, the samples can be processed in a standard manner for electron microscopy.

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Possible problems and their solutions

Weak staining:

- Increase the concentration of injected Lucifer Yellow CH.
- Increase the volume of dye injected by microinjection.
- Increase the dye delivery time using iontophoresis or electroporation methods.
- Enhance staining using immunochemistry with antibodies specific to Lucifer Yellow.

Weak labeling of processes:

- Ensure that the concentration of Lucifer Yellow CH is not too high. Excessive dye concentration can lead to aggregation and reduced diffusion.
- Check the electroporation or microinjection parameters to ensure efficient dye delivery.
- Increase the dye diffusion time before fixation.
- Optimize the fixation and permeabilization steps to avoid quenching of fluorescence or blocking of dye penetration into the cell processes.

References

[1] J. Cell. Mol. Med. 2012. 16(1). pp. 22-31.

[2] Science. 1982. 217. pp. 953–955.

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