

Mouse Calcein and Alizarin Protocol

Calcein (Sigma C0875):

1. Add 0.4g Sodium bicarbonate (Sigma S5761) to a 10mL bottle of 0.9% NaCl (buy at DCM Rx, bottle with green cap)
2. Add 0.05 g of calcein green to the bicarb/NaCl solution in a 50mL conical tube.
3. Add more 0.9% NaCl to a **FINAL VOLUME of 20ML**
4. Use a 20cc syringe and an 18-gauge needle to transfer all of the solution into the syringe.
5. Replace the needle with a 22µm syringe filter.
6. Filter the calcein solution into a new 50mL conical tube.
7. Cover the tube with aluminum foil.
8. Label and store in refrigerator at 4°C. Solution should be good for up to 3 months and 200 mice.
9. Aliquot what you need for each experiment in the tissue culture hood to keep stock sterile.
10. Warm solutions with hand or let sit at RT (still covered) before injection.

Label on calcein solution should include:

- Date solution was made
- “Mouse Calcein”
- **Injection volume: 0.1mL/25g whole body weight**
- **Dose: 10 mg/kg**

Alizarin (Sigma A3882):

Use the same protocol as above, but make the following substitutions:

- 0.15 g Alizarin (not 0.05 g calcein in Step 2)
- “Mouse Alizarin” on label
- **Injection volume: 0.1mL/25g whole body weight**
- **“Dose: 30 mg/kg” on label**

If calcein/alizarin doesn't go into solution immediately, there is something wrong.

-Always use fresh powder

-Try to be as exact with addition of sodium bicarbonate as possible.