10 X Sucrose Orange G Loading Dye

Gordon Wellman

Abstract

This protocol outlines the preparation of a basic sucrose loading dye 10 X stock solution for gel electrophoresis (DNA/RNA). Orange G runs at approximately 50 bp making this loading dye ideal for small molecular weight polynucleotides. Used mainly as an indicator for approx. 100 bp polynucleotides, where we would like to avoid contamination of small fragments with other loading dyes. It is also useful as an indicator if you have excessive primer-dimer amplification in your PCRs as these usually also run at approx. 50 bp.

The origins of this protocol have been lost in the mists of time and this method been handed down from PhD student to PhD student.

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Materials

- Sterile water by Contributed by users
- Sucrose by Contributed by users
- Orange G, sodium salt by Contributed by users

Protocol

Final Concentrations

Step 1.

Orange G, sodium salt	**0.4 % (w/v)**
Sucrose	**40 % (w/v)**

Loading Dye 10 X stock preparation volumes

Step 2.

Final volume	25 mL	50 mL
orange G, sodium salt	0.05 g	0.1 g
sucrose	10 g	20.0 g
sterile water	to 25 mL	to 50 mL

Preparation

Step 3.

Dissolve sucrose entirely into a smaller (20 or 40 mL) than total volume of warm sterile water.

Step 4.

Allow to cool to room temperature.

Step 5.

Add Orange G, sodium salt, top up with sterile water to final volume.

Step 6.

Dispense 1 mL aliquots into labelled 1.5 mL Eppendorf tubes for ease of use later.

Step 7.

Store loading dye at -20 °C until use. Keeps very well (for years at -20 °C).

Use

Step 8.

Ensure loading dye is fully defrosted before use or it will become very difficult to handle. (This is why the 1.5 mL aliquots are best practice, otherwise, it takes too long to defrost and will be like pipetting a syrup)

Use 1 µL of 10 x loading dye per 10 µL of PCR product for gel electrophoresis.

Loading dye can be re-frozen if the full aliquot is not used at once, without any noticeable effect on following samples.