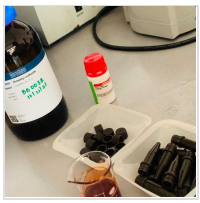

Apr 29, 2022

SOP024: Preparing 10000x DNA gel stain

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protocol .

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DNA dyes stain deoxyribonucleic acid for laboratory purposes such as detection and quantification. Many DNA dyes also bind to RNA and could be more broadly described as nucleic acid stains. Common dyes included ethidium bromide (EtBr), especially for agarose gel electrophoresis of DNA.

DNA gel electrophoresis using agarose is a common tool in molecular biology laboratories, allowing separation of DNA fragments by size. After separation, DNA is visualized by staining.

Thiazole orange compares favorably to common staining methods in that it is sensitive, inexpensive, excitable with UV or blue light (to prevent sample damage), and safer than ethidium bromide.
Thiazole orange is known to interact with DNA and produce significant fluorescence which can be used to visualize DNA in electrophoresis gels.
Thiazole orange powder can dissolve completely without precipitating in several solvents except water.

Scope:

This protocol covers the steps involved in making a 10000x stock DNA gel stain using Thiazole orange dye powder.
This protocol also covers the steps involved in using the 10000x stock to prepare a 1.3ug/ml final concentration of gel stain required to visualize DNA bands on an agarose gel.

The solvent used for this protocol is DMSO.

Shalo Minette, Stephane Fadanka, Nadine Mowoh 2022. SOP024: Preparing 10000x DNA gel stain. **protocols.io** https://protocols.io/view/sop024-preparing-10000x-dna-gel-stain-b5ewq3fe

Thiazole Orange, DNA gel stain

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Feb 21, 2022

Apr 29, 2022

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It is advisable to prepare the DNA gel stain in large batches(10-15 tubes) to enable easy weighing of the powder especially in situations where a high precision balance is unavailable.

- Thiazole Orange Dye powder
- DMSO
- Weighing balance
- Weighing boat
- Spatula
- Brown/Black Scroll cap microcentrifuge tubes
- Eppendorf tubes
- Refrigerator
- 1000µl Micropipette
- 1000µl Pipette tips

- Weigh safety googles, gloves and face masks before weighing out the dye powder.
- Avoid the formation or emission of dust particles.

- Make sure to assemble and keep ready all materials, equipment and reagents needed for the procedure.
- Check to be sure the weighing balance and Micro pipette is working correctly (are able to weigh the right mass in the right units, and picks up the right volume respectively).

Preparing a 13mg/ml stock of DNA gel stain (1ml) 5m

1 Use a weighing balance to carefully measure **0.013 g** of Thiazole Orange powder (Cas # 107091-89-4) into a 1.5ml Eppendorf tube or opaque screw cap tube. 3m

2 Use a micropipette to pipette **1000 µL** of DMSO (Cas # 67-68-5) into the same Eppendorf tube. 1m

3 Mix by gently inverting the tube several times to have a 10000x stock. This step may take a minute or less. **00:01:00** 1m

4 For immediate or daily use, leave at room temperature away from contact with sunlight (It can be stable up to 3 months. If not immediately used, store the gel stain stock in the fridge or freeze for 6 to 12 months.

Preparing a 1.3ug/ml final concentration of DNA gel stain for agarose gel electrophoresis 52m

5 Follow the step 9 in this protocol to prepare a 1X TBE buffer from a 10X stock 2m

6 Prepare a 2% agarose gel by diluting 0.5g of agarose powder in 25ml 1x TBE or (calculate the corresponding amount of agarose to use for the percentage and volume of agarose gel desired). 30m

> The percentage of your gel would depend on the size of DNA template to be amplified and the volume of gel on the size of the casting tray available.

7 From the 10000x DNA gel stain stock, pipette **2.5 µL** into **25 mL** molten agar

8 Cast the gel and leave to polymerize on the working bench at **Room temperature** . This may take about **00:20:00** . 20m

9 Load and run the gel immediately or you can keep the gel on the bench at room temperature till you are ready (the gel can stay up to 3 to 6 hours without affecting visualization of DNA bands).