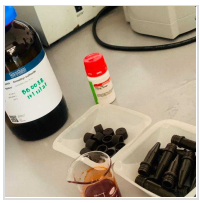

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Jun 14, 2022

Production of 10000x TO-DMSO DNA gel stain V.2

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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. Production of 10000x TO-DMSO DNA gel stain. **protocols.io** https://protocols.io/view/production-of-10000x-to-dmso-dna-gel-stain-cabnsame Nadine Mowoh

Beneficial Bio Product: 10x TBE Buffer Sachets

Added quality control tests for the DNA gel stain

Thiazole Orange, DNA gel stain

protocol ,

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Part of collection

Beneficial Bio Product: 10x TBE Buffer Sachets

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 **Sigma – Aldrich**
- Dimethyl Sulfoxide (DMSO) **Contributed by users**
- 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 #) Sigma – Aldrich** into a 1.5ml Eppendorf tube or opaque screw cap tube. 3m

2 Use a micropipette to pipette **1000 µL** of **Dimethyl sulfoxide (DMSO) Contributed by users** 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

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.

To prepare more than 1ml gel stain (say 10-15ml), calculate the amount of Thiazole orange powder needed for the final volume of gel stain desired.

- Weigh the powder and pour into a 50ml beaker, measure DMSO and add to the powder.
- Swirl gently for 1min to completely dissolve the powder.
- Transfer the gel stain into desired storage tubes by pipetting the desired volumes for each tube.

O'Neil CS, Beach JL, Gruber TD (2019). DNA Electrophoresis Using Thiazole Orange Instead of Ethidium Bromide or Alternative Dyes.. Journal of visualized experiments : JoVE. https://doi.org/10.3791/59341

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

4 Follow this step to prepare a 1X TBE buffer from a 10X stock 20m

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).

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

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

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).

Quality control tests for TO-DMSO gel stain

5 The following quality control tests are performed after producing the 10000x DNA gel stain to confirm it meets its desired specifications.

Functionality test

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The functionality test is carried out to ensure the gel stain is able to allow visualization of amplified DNA templates after an agarose gel electrophoresis. This is done by performing gel agarose electrophoresis of any desired DNA template. Our lab adopted the Lambda genome using the 0.5 and 1kb regions for this test.

This is done in 3 steps:
1. Amplification of DNA template in a PCR reaction
2. Running of Agarose gel electrophoresis
3. Visualization of gel

7 **Amplification of DNA template in a PCR reaction**

Thaw all reagents on ice in a bowl

Label reaction tubes (PCR tubes) according to the number of samples, and including controls in each run (negative and positive controls) as needed.

If using a 10x cellular reagent enzyme- Rehydrate the enzyme with 30µl of sterile PCR grade water, flick tube and keep on ice.

Enzyme Master mixes can be used if available. In our case we used OpenVent DNA polymerase.

8 In each PCR tube, pipette and combine the following reagent components following the order in the table below (work on ice while pipetting)

A	B	C	D
Component	**Test Sample**	**Negative Control**	
PCR Water	Variable up to 20ul	Variable up to 20ul	
10x PCR buffer	2ul	2ul	
10mM dNTP Mix	0.4ul	0.4ul	
Forward/ Reverse primer	1/1	1/1	
DNA template (0.5/1kb Lambda)	0.5-1ul	/	
DNA polymerase enzyme (OpenVent)	1ul	1ul	

9 Gently mix the reaction tubes by flicking and transfer in the PCR machine to begin thermocycling.

10 Connect the thermocycler and program the protocol to run following the thermocycling steps listed below. The exact annealing temperature will depend on the choice of DNA template and primers used.

A	B	C
Initial Denaturation	95°C	120secs
Denaturation	95°C	30sec
Annealing	50-65°C (depending on the length of the primers)	20secs
Extention	72°C	Variable depending on primer
Final extention	72°C	120secs

11

The time of run will depend on the size of the DNA template and number of cycles of amplification (usually we use do 35 to 40 cycles).

12 **Preparing and running agarose gel electrophoresis** 30m

About **00:30:00** to the end of the thermocycling process, prepare a 1x TBE buffer from 10x stock following this protocol and use it to make a 2% agarose gel following this protocol.

At this step a positive control gel a can be prepared using an EtBr based gel stain, which will be used to compare the results (band quality) of the TO-DMSO gel stain.

The two gels are then run under the same conditions and the resulting DNA bands visualized and compared.

13 **Loading the agarose gel wells**

Pipette 3 to 5µl of DNA ladder (size depending on size of the DNA template) unto the first well then pipette the negative control carefully to avoid bubbles and load into the second well.

Following the step as described here, mix your amplicons with DNA loading dye and load in the subsequent wells

Allow the gel to run for 15-30mins at 48V on 0.8 or 2% agarose gel and TBE buffer

14 **Visualization**

The amplification results are visualized by transferring the gel into a UV transilluminator or blue gel transilluminator

With the help of the DNA ladder, determine if the amplification product is of the expected size and the bands are sharp and distinct looking exactly like those from the EtBr gel to show that the TO-DMSO gel stain was able to stain the DNA.

Confirm visualization of low molecular weight DNA

15

Determined by 1.5% TBE Agarose gel electrophoresis of 50 or100bp Ladder

Follow the steps in preparing a 2% agarose gel and load as described in step 17 above. DNA templates or amplicon in this case is a 100bp DNA ladder.
Run gel to finish and visualize the resulting band separation to confirm separation of the ladder into visible distinct bands .

Confirmation of visualization of low concentration of DNA

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Determined by 1.5% TBE Agarose gel electrophoresis of serial dilutions of 100bp Ladder

Make 1:5 serial dilutions of the 100bp DNA ladder or any DNA amplicon and use the different dilutions as amplicons to load a gel well.
Allow the gel to run through and visualize to determine the lowest concentration detectable by the gel stain.

At this stage, the gel stain is ready to be used either on the spot or can be shipped or sent to an external lab and used following the steps described in the user protocol or instructions in step 4 above.