Jun 24, 2022

Production of 6x DNA Loading Dye

In 1 collection

Stephane Fadanka[1], Nadine Mowoh[1]

[1]Beneficial Bio, Mboalab

1 *Works for me* Share

This protocol is published without a DOI.

Nadine Mowoh

ABSTRACT

The process of gel electrophoresis involves separating DNA fragments using an electrical current while tracking the rate of molecular movement through a filtering gel. Adding blue or orange tracking dye to colourless DNA samples allows you to see your sample and obtain information about how DNA molecules move during electrophoresis. Identification is based on the size of DNA bands on the gel after migration of molecules.

The Beneficial Bio 6x loading dye is composed of bromophenol blue and xylene cyanol. Thus, it helps you determine the rate of movement of different size DNA molecules in the gel during electrophoresis.
When composing or preparing DNA loading dyes, a heavy, syrupy substance that gives more density to the DNA sample before it is inserted in the wells is needed. This will help the DNA sample sink forming a layer in the well instead of being dispersed in the absence of a heavy substance. In this formulation, trehalose is used to give the DNA samples the desired viscosity.

PROTOCOL CITATION

Stephane Fadanka, Nadine Mowoh 2022. Production of 6x DNA Loading Dye. **protocols.io** https://protocols.io/view/production-of-6x-dna-loading-dye-cbdcsi2w

COLLECTIONS

Beneficial Bio Products

KEYWORDS

preparing 6x DNA loading dye, preparing DNA tracking dye

CREATED

Jun 15, 2022

LAST MODIFIED

Jun 24, 2022

PROTOCOL INTEGER ID

64644

PARENT PROTOCOLS

Part of collection
Beneficial Bio Products

MATERIALS TEXT

Trehalose **Contributed by users** Step 1 in a final concentration of 14.48%

SDS **Contributed by users** Step 2 in a final concentration of 0.3%

Na2EDTA **Sigma – Aldrich** Step 3

Glycerol **Sigma – Aldrich** in a final concentration of 23.4%

Xylene cyanol **Bio Basic Inc.** Step 4

Bromophenol blue **Bio Basic Inc.** Step 4

Electronic or sensitive weighing balance
Measuring cylinder
15ml Eppendorf tube

SAFETY WARNINGS

All reagents and chemicals used here are generally safe but it is advisable to always wear protective clothing to avoid accidental spills or splashes.

BEFORE STARTING

Check to make sure you have all the starting materials available and assembled to prepare the reagent stocks.

Preparation of Reagent stocks 10m

1

In order to produce a 6x DNA loading dye, we begin by preparing reagent stocks and weighing dye powders to constitute a concentrated dye stock (could be called a 60x stock) which is later diluted to make the 6x Dye stock.

Preparing [M]20 % (v/v) Trehalose (100ml)

- Accurately weigh **20 g** of Trehalose **Contributed by users** powder into a **500 mL** beaker
- Use a measuring cylinder to measure **100 mL** of sterile distilled water into the beaker and stir gently until the powder is completely dissolved.
- Transfer the content into a clean Duran bottle, cork and keep at **4 °C** until used.

2 **Preparing [M]10 % (v/v) SDS Contributed by users (100ml)**

- Accurately weigh **10 g** of SDS powder into a **500 mL** beaker
- Use a measuring cylinder to measure **100 mL** of sterile distilled water into the beaker and stir gently until the powder is completely dissolved.
- You may heat it up a little up to 40c to 60c to ease dissolution but do not autoclave as it may result in precipitation.
- Transfer the content into a clean Duran bottle, cork and store at **Room temperature** or **4 °C** until used.

3 **Preparing [M]0.5 Molarity (M) Na2EDTA Sigma – Aldrich (100ml)**

- Weigh out **18.61 g** EDTA disodium salt, dihydrate and add to a 100 mL Duran bottle.
- Measure out **80 mL** distilled water and add to the Duran bottle.
- Add a magnetic flea and place on a magnetic stirring plate to mix the solution. The EDTA salt will not go into solution until the **pH 8**.
- Add a pH meter into the solution to observe the pH.

This step may be necessary only when there is EDTA powder available.
For those who have 0.5M EDTA pH 8 they may skip this step and just pipette the required volume of solution to reconstitute.

Weighing the dye powders and reconstituting the concentrated (60x) dye stock 10m

4

This protocol is suited for 10ml concentrated dye stock but can be adjusted for the desired volume.
The concentrated stock is later diluted 10x to give a 6x working stock.

- With the help of a weighing balance, weigh accurately **0.015 g** of Bromophenol blue **Bio Basic Inc.** into a 15ml Eppendorf tube
- Weigh **0.015 g** of Xylene cyanol **Bio Basic Inc.** into the same tube
- From a 20%w/v Trehalose stock, aliquot some volume into the Eppendorf tube to dissolve the dye powder.
- Into the same tube, add **120 µL** of **0.5 Molarity (M)** EDTA
- Measure **300 µL** of **10 % (v/v)** SDS into the same tube
- Measure **2.34 mL** of **100 % (v/v)** Glycerol **Sigma – Aldrich** and add into the tube
- Finally add in bits 20% Trehalose to make up the volume to **10 mL**
- Cork tightly the Eppendorf tube and mix the content gently to homogenise
- Label the tube and either store it at room temperature or **4 °C** for up to 12 months.

For longer periods, store at **-20 °C**

Preparing 6x DNA loading dye (working stock) 2m

5 **1:10 Dilution from the concentrated stock**

- From the dye stock, pipette **166.6 µL** into the Eppendorf tube then add **833.3 µL** of **20 % (v/v)** Trehalose to make a 1ml working solution (6x) of the loading dye. **(Or Pipette 100 µL from the concentrated dye stock and add 900 µL of 20 % (v/v) Trehalose).**
- Cork the tube firmly and mix by inverting the tube several times.

- Store the working solution at room temperature or **4 °C** for up to 12months.

For longer periods, store at **-20 °C**

Quality control tests

6 The Quality control tests performed for the DNA loading dye include:
- Functionality test
- Nuclease test

The protocols can be found here.

> We adopted these tests to confirm the integrity and quality of our DNA loading dye formulation but the quality control tests that could be performed are not limited to these two.

Packaging

7 If the DNA loading dye is not used on the spot or is to be sent outside the facility, it is packaged under aseptic conditions to reduce or control contamination that may affect its functionality following the steps in **this protocol**.

User protocol and trouble shooting

8 During packaging, a user manual or datasheets are included to help the user effectively use the product. Before using the DNA loading dye, read and follow the instructions described in the user protocol.

If any difficulty or abnormalities are encountered during production or using, refer to the **trouble shooting** section of the user protocol.