Electrophoresis Dyes

Posted by Anonymous on Fri, 2015-12-04 17:06

Electrophoresis Dyes: Hi, We have recently purchased our own equipment for doing electrophoresis and would like to know what the best dyes to use are and their preparation please?

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Laboratory Technicians

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Electrophoresis Dyes

Submitted by on 11 December 2015

Answer reviewed 22 February 2023

The electrophoresis of food dyes and scientific stains in school science laboratories demonstrates the basic principles and procedures of gel electrophoresis in a simple way. The dyes and stains used are affordable, safe, and the procedure simpler than using DNA. A big advantage is that they behave in a similar way to DNA molecules during electrophoresis, and any bands produced can be visualized directly in the gel, without any further staining.

Different scientific stains produce distinct bands of colour during electrophoresis, whilst food dyes can be separated into their different pigments.

Samples of stains or food dyes are required to be mixed with a 20% v/v glycerol/distilled water solution prior to electrophoresing. The glycerol will make the sample denser than the electrophoresis buffer and allow the sample to sink and remain in the well rather than float into the buffer. This is an important step, so that no dye floats into the buffer and cross contaminates other samples.

Samples can be loaded into the gel using micropipettes, transfer or Pasteur pipettes. Care needs to be taken not to puncture the bottom of the well or overfill the wells. Loading every second well is also a good idea to minimise the chance of contamination between adjacent wells. A 10–20 uL (1drop) sample size is generally used and the samples are electrophoresed at 90–100 volts for 30–35 min.

Preparation of samples

Food dyes can be purchased at the supermarket. They are negatively charged molecules like DNA molecules and will migrate to the anode (+ve terminal).

Preparation of food dyes for electrophoresis

1. Prepare 2 mL of a 20% v/v glycerol/distilled water solution.
2. Using a transfer or automatic pipette, add 0.1–0.2 mL of food dye to the glycerol solution and mix thoroughly until the dye is evenly distributed.
3. Repeat steps 1 and 2 for each colour.
4. Store the prepared dye solutions at 4° C for a longer shelf life.

Electrophoresis of food dyes will result in the following pigment separations.

- Green = blue and yellow bands
- Red = pink and red/orange bands
- Yellow = pink, orange and yellow bands
- Blue = light blue, dark blue and dark red bands
- Black = orange/red, yellow, orange, pink, light blue and dark blue

Scientific stains such as Bromophenol Blue[1], Tartrazine[2], Rose Bengal[3], Xylene Cyanol[4], and Orange G[5] are commonly used. These can be purchased from various scientific suppliers as ready-to-use reagents or can be made up from individual powdered stains at a concentration of 0.2% w/v in 20% glycerol/distilled water. NB Bromophenol Blue is not soluble in water. Dissolve in a small amount of ethanol before diluting in water. Stains can be used as a mixture, often referred to as a ‘ladder’. They produce distinct bands of colour when electrophoresed.

Most scientific stains are negatively charged and will migrate towards the anode (+ve terminal). Methyl Green is positively charged and will migrate towards the cathode (-ve terminal). The smaller the molecule the further it will migrate.

Preparation of stain solutions for electrophoresis

1. Prepare 10 mL of a 20% v/v glycerol/distilled water solution.
2. Add 20 mg of the required stain to the glycerol solution and mix thoroughly until the powder has completely dissolved. This may take a while.
3. Store the prepared stain solution in a brown bottle at room temperature to increase shelf life (slowing decomposition).
4. If requiring a mixture, then combine prepared stains in a 1:1 ratio for use.

Some safety and other considerations

- The power supply and the gel apparatus use high voltage and should be handled with caution. Ensure electrodes are in place before turning on. Make sure the power supply is turned off before disconnecting any leads and when not in use.
- Do not operate the power supply in damp or humid conditions. Keep water away from the power source.
- Gloves and eye protection should be worn at all times during the procedure.
- Observe the position of the bands during the electrophoresis procedure, so that it can be stopped when sufficient separation has occurred and to make sure that the bands do not run off the end of the gel.
- Measurements and photographs should be taken soon after stopping the electrophoresis as the molecules will continue to diffuse into the gel.
- If using samples that will migrate to both the anode and cathode, the wells should be prepared in the centre of the gel to allow good visualisation of the stains as they move to the different electrodes.
- At the end of the experiment, place all used gels in a plastic bag and dispose in the general waste bin. Used buffer solutions can be disposed of down the sink.
- Safety Data Sheets (SDSs) should be consulted as a first step in assessing the risks associated with a chemical: most of the stains used during electrophoresis are irritants.

Additional information

Gel electrophoresis

Gel electrophoresis is the separation of molecules through their migration in an electric field. The molecules are separated based on their size, electrical charge and shape when applied to a gel medium. Electrophoresis is a commonly used technique in laboratories to analyse DNA, RNA and protein samples, plus other compounds in mixed samples. The principle of electrophoresis is that molecules are placed into a gel that is subjected to an electric current. Any negatively charged molecules will move towards the anode (+ve terminal) , whilst positively charged molecules will move towards the cathode (-ve terminal). The smaller the molecule, the further it will migrate. The density of the gel and the type of buffer used will affect how a molecule migrates. Most biological molecules are electrically charged, causing them to move when subjected to an electric field.

References

Southern Biological (nd) '*Gel Electrophoresis of dyes*', Retrieved (22 February 2023) from the Southern Biological website: https://www.southernbiological.com/dyes-for-electrophoresis/

Southern Biological (nd) '*Biotechnology: Getting started with Gel Electrophoresis*', Retrieved (22 February 2023) from the Southern Biological website: https://www.southernbiological.com/getting-started-with-gel-electrophore...

Safety data sheets:

1Chem-Supply. (2019) *Bromophenol Blue,* Safety Data Sheet. Search https://shop.chemsupply.com.au/ to source the latest Safety Data Sheet via the product information page.

2Chem-Supply. (2018) *Tartrazine Yellow,* Safety Data Sheet. Search https://shop.chemsupply.com.au/ to source the latest Safety Data Sheet via the product information page.

3Sigma-Aldrich. (2022) *Rose Bengal,* Safety Data Sheet. Search https://www.sigmaaldrich.com/AU/en/sds/aldrich/330000 to source the latest Safety Data Sheet via the product information page.

4Sigma-Aldrich. (2020) *Xylene Cyanol FF,* Safety Data Sheet. Search https://www.sigmaaldrich.com/AU/en/sds/sigma/x4126 to source the latest Safety Data Sheet via the product information page.

5Sigma-Aldrich. (2022) *Orange G,* Safety Data Sheet. Search https://www.sigmaaldrich.com/AU/en/sds/sigma/o3756 to source the latest Safety Data Sheet via the product information page.

Electrophoresis Dyes

Submitted by on 08 December 2015

We found it easier to buy the dyes from Southern Biological.

Electrophoresis Dyes

Submitted by VIC003 on 10 February 2025

What is the buffer that is used with this food dye method please? Is the buffer used in making the agar gel? Thank you.
