

HiPer[®] Agarose Gel Electrophoresis Teaching Kit

Product Code: HTBM001

Number of experiments that can be performed: 10

Duration of Experiment: 1 hour

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Genomic DNA, Plasmid DNA & 1 kb DNA Ladder at -20°C
 - Store 6X Gel Loading Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)



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Aim:

To visualize separated DNA fragments according to their molecular size by applying an electric current to the gel matrix.

Introduction:

Agarose gel electrophoresis is a method used to separate DNA and RNA molecules according to their molecular size. This is achieved when negatively charged nucleic acids migrate through an agarose gel matrix under the influence of an electric field (electrophoresis). Shorter molecules with low molecular weight move faster and migrate farther than the larger ones. This method is simple, rapid to perform, and capable of resolving fragments of DNA that cannot be separated by other procedures such as density gradient centrifugation. The position of DNA in the agarose gel is visualized under UV light by staining with low concentration of fluorescent intercalating dyes, such as Ethidium bromide.

Principle:

Agarose is an unmodified polysaccharide of galactose with neutral charge, which is essential to prevent interactions with charged DNA and protein molecules. It forms large pores which is useful for separation of DNA and proteins by its molecular size. In aqueous solution, below 35°C these polymer strands are held together in a porous gel structure by non-covalent interactions like hydrogen bonds and electrostatic interactions. On heating the solution, these non-covalent interactions are broken down and the strands are separated. As the solution cools, these non-covalent interactions are re-established and the gel is formed. Purified agarose is insoluble in water or buffer at room temperature but dissolves on boiling. As it cools, agarose undergoes polymerization i.e., sugar polymers cross-link with each other and cause the solution to solidify, the density or pore size of which is determined by concentration of agarose.

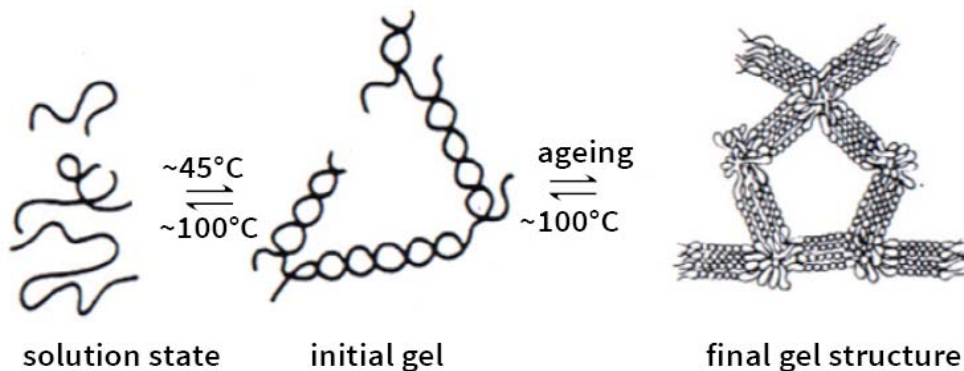


Fig 1: Different states of agarose gel formation depending upon the temperature

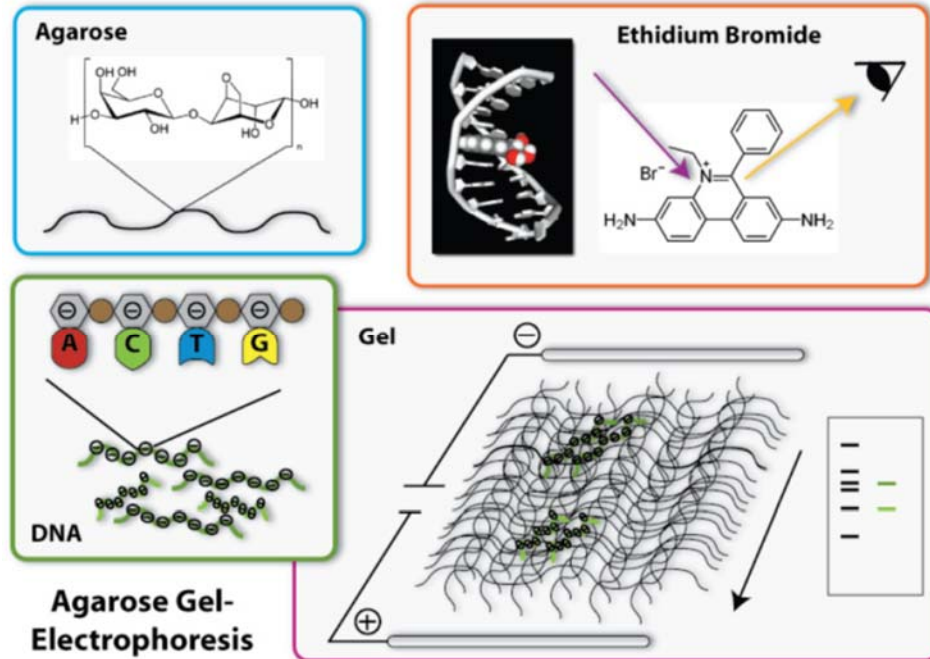


Fig 2: Negatively charged DNA molecules move through the agarose gel with the application of electric current and can be visualized by using ethidium bromide which fluoresces under the ultraviolet light

The following factors determine the rate of migration of DNA through agarose gels:

- **Molecular size of DNA:** Fragments of linear DNA migrate through agarose gel with a mobility that is inversely proportional to the \log_{10} of their molecular weight. Larger molecules migrate more slowly because of greater frictional drag as they pass through the pores of the gel less efficiently than the smaller molecules. Circular forms of DNA migrate in agarose differently from linear DNA of the same mass. Undigested plasmids migrate more rapidly than the same plasmid when linearized.
- **Agarose Concentration:** By using gels with different concentrations of agarose, DNA fragments of different sizes can be resolved. Higher concentration of agarose facilitates separation of small DNA, while lower agarose concentration allows resolution of larger DNA.
- **Electrophoresis buffer:** Several buffers have been recommended for electrophoresis of DNA. The most commonly used are TAE (Tris-acetate-EDTA) and TBE (Tris-borate-EDTA). DNA fragments will migrate at different rates in these two buffers due to difference in ionic strength. Buffers not only establish a pH, but also provide ions required to support conductivity.
- **Effect of Ethidium bromide:** Ethidium bromide is a fluorescent dye which fluoresces at 254–366nm. It intercalates between bases of nucleic acids and allows very convenient detection of DNA fragments in gels on a UV transilluminator. Binding of ethidium bromide to DNA alters its mass and rigidity, and therefore its mobility.
- **Voltage:** As the voltage applied to a gel is increased, larger fragments migrate proportionally faster than smaller fragments. The best resolution of fragments larger than about 2 kb is attained by

applying voltage not more than 5 volts per cm to the gel (the cm value is the distance between the two electrodes, and not the length of the gel).

Kit Contents:

This kit can be used to visualize DNA by agarose gel electrophoresis.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			10 expts	
1	TKC001	Genomic DNA	0.11 ml	-20°C
2	TKC002	Plasmid DNA	0.11 ml	-20°C
3	MB002	Agarose	4.8 g	R T
4	ML016	50X TAE	120 ml	R T
5	MBT051	1 kb DNA Ladder	0.035 ml	-20°C
6	ML015	6X Gel Loading Buffer	0.06 ml	2-8°C

Materials Required But Not Provided:

Glasswares: Conical flask, Measuring cylinder, Beaker

Reagents: Distilled water, Ethidium bromide (10 mg/ml)

Other requirements: Electrophoresis apparatus, UV Transilluminator, Micropipettes, Tips, Adhesive tape, Microwave/Burner/Hotplate

Storage:

HiPer® Agarose Gel Electrophoresis Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store 1 kb DNA Ladder and samples at -20°C and the 6X Gel Loading Buffer at 2-8°C. Other kit contents can be stored at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. **Preparation of 1X TAE (gel electrophoresis buffer):** To prepare 500 ml of 1X TAE buffer, add 10 ml of 50X TAE Buffer to 490 ml of sterile distilled water*. Mix well before use.
* Molecular biology grade water is recommended (Product code: ML024).

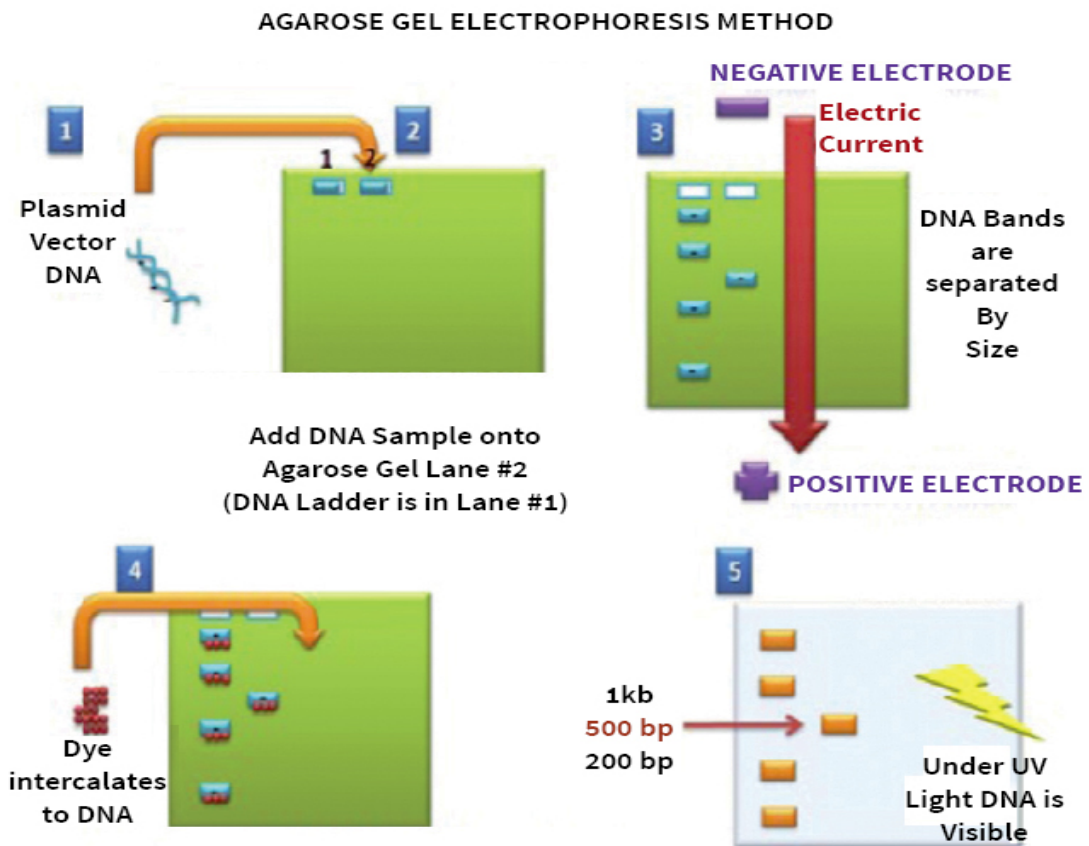
Procedure:

1. Prepare gel tray by sealing the ends with adhesive tape. Place comb in the space provided on the gel tray.
2. To prepare 50 ml of 0.8 % agarose solution, add 0.4 g agarose to 50 ml 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave/burner/hot plate by swirling the glass beaker/ flask

occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure).

3. Allow solution to cool down to about 55-60°C. Add 0.5 µl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.
4. To start the run, carefully remove the adhesive tape from both the ends of the gel tray, place the tray in electrophoresis chamber, and fill the chamber (just until wells are submerged) with 1X TAE electrophoresis buffer and gently remove the comb.
5. To prepare samples for electrophoresis, add 2 µl of 6X gel loading buffer for every 10 µl of DNA sample. Mix well and load the sample into the well. Load 3 µl of 1 Kb DNA Ladder into one of the wells.
6. Connect the power cord to the electrophoretic power supply according to the conventions: Red- Anode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.
7. Electrophoresis apparatus should always be covered with the lid to avoid electric shocks. Avoid use of very high voltage as it can cause trailing and smearing of DNA bands in the gel, particularly with high molecular weight DNA.
8. Switch off the power supply once the tracking dye from the well reaches 3/4th of the gel which takes approximately 45 minutes.

Flowchart:



Safety:

➤ Precaution:

UV light can damage the eyes and skin. Always wear suitable eye and face protection while working with a UV light source. UV light damages DNA. If DNA fragments are to be extracted from the gel, use a lower intensity UV source if possible and minimize exposure of the DNA to the UV light.

➤ Disposal of Ethidium bromide waste:

All items that were in contact with Ethidium bromide must be disposed off in the designated waste container (marked with "Ethidium bromide waste") within the Gel-Doc-Area, including gels, tissue paper to clean UV table, and nitrile gloves.

➤ Hazard:

Ethidium bromide is a powerful mutagen and is very toxic. Appropriate safety precautions should be taken by wearing latex gloves; however, use of nitrile gloves are recommended.

Observation and Result:

The illumination of a stained gel under UV light (254–366 nm) allows DNA bands to be visualized against a background of unbound dye. The gel image can be recorded by taking a Polaroid™ photograph or using a gel documentation system.

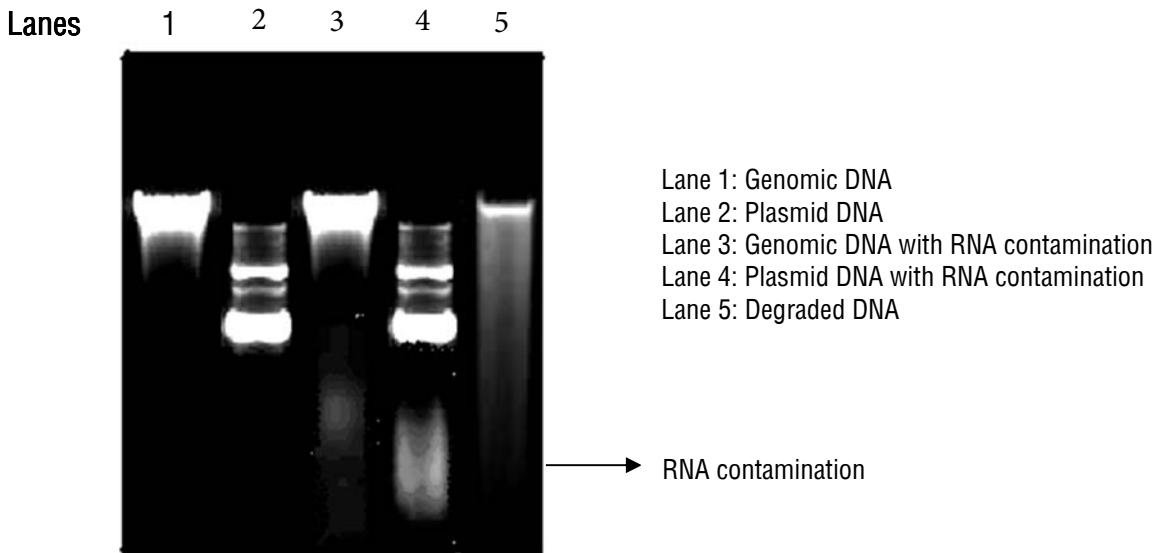


Fig 3: Genomic and plasmid DNA under UV light after gel electrophoresis

Interpretation:

Agarose gel electrophoresis and spectrophotometric analysis will confirm the yield and purity of DNA. Genomic DNA being of larger molecular size migrates at a lower speed than the plasmid DNA of smaller molecular size. If RNA contamination is present, one would see a faint and smeary RNA band below the genomic and plasmid DNA since RNA being of lower molecular weight runs faster than the genomic and plasmid DNA.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Faint or no band seen on the gel	Insufficient quantity of DNA loaded on the gel	Load exact amount of DNA as per the procedure
		Inaccurate amount of Ethidium bromide added to the gel	Always add proper amount of Ethidium bromide as given in the procedure
		DNA electrophoresed off the gel	Electrophorese the gel for less time, use a lower voltage. Do not allow the gel loading dye to run off the gel
		DNA does not run in the proper direction	Always connect the cords of the electrophoretic unit as per convention: Black- Cathode, Red- Anode
2	Smeared DNA bands	DNA was degraded	Avoid nuclease contamination
		Improper electrophoresis condition	Ensure that the gel tray is submerged in electrophoresis buffer
		Gel Electrophoresis Buffer reused several times	Prepare fresh TAE buffer, dilute it as per instructions
3	Improper gel solidification	Gel not melted properly	Prepare gel as per the procedure. Ensure that the agarose is dissolved
		After preparation, gel kept for too long at room temperature	completely before pouring into the gel tray Add Ethidium bromide at right temperature after gel preparation and immediately pour the gel. Do not let the gel to solidify before pouring

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com



Storage temperature



Do not use if package is damaged



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