Bio Bharati Life Science Pvt. Ltd.

Agarose Gel Electrophoresis

Cat# BB-MTK060 (10 reactions)

Aim: To visualize the separation of different DNA fragments (provided in the kit) of different molecular weights by using agarose gel electrophoresis.

Principle: Agarose Gel Electrophoresis is a method to separate nucleic acid (DNA or RNA) in agarose matrix. It can be used to separate mixed population of DNA or purified plasmid or genomic DNA. The technique is the most popular and has been used widely in the field of biochemistry, molecular biology, genetics and clinical chemistry.

Agarose is a polysaccharide extracted from seaweed. It is typically used at concentrations of 0.5 to 2%. Higher agarose concentration is used to separate lower molecular size DNA and lower agarose concentration for high molecular weight DNA. For separation of RNA higher concentration of agarose is mainly used.

Agarose gels have a large range of separation, but relatively low resolving power. The gel is cast in the shape of a thin slab, with wells for loading the samples. The gel is immersed within an electrophoresis buffer that provides ions to carry current and maintain the pH at a relatively constant value. When DNA/RNA molecules are placed in an electric field, due to their phosphate backbone they possess the negative charge and migrate towards the anode.

Kit Description: In this kit, two different DNA samples of different sizes along with the known DNA standard are supplied. Students will prepare a 0.7% agarose gel mixed with ethidium bromide (following the protocol provided in the kit) and run two DNA samples along with the known standard DNA Molecular Weight Marker. The separated DNA upon visualization under UV light will give an indication of the purity of the unknown test DNA samples. Molecular weight of the unknown DNA will also be determined by comparing its relative mobility with that of the known standard. The DNA Molecular Weight Marker has molecular weights in the range of 500-12000 bp.

#BB-MTK060: The kit is designed to carry out 10 agarose gel electrophoresis experiments.

Duration of experiment: Approximately 3-4 hours.

Kit Contents:

Sl No	Items	Quantity for 10 reactions	Store at
1	Agarose	2gm	RT
2	Ethidium Bromide	25µl	RT
3	50X Running Buffer	30ml	RT
4	6X Loading Dye	50µl	4˚C
5	Molecular Weight Marker	50µl	-20 ˚C
6	DNA Sample 1	50µl	4˚C
7	DNA Sample 2	50µl	4˚C

Materials required but not provided:

Consumables: Measuring cylinder, Conical flask, Beaker
Reagents: Distilled water.
Instrument: Horizontal gel electrophoresis unit, UV Trans-illuminator, Micro tips, Adhesive tape, Hot-plate or Bunsen burner.

Storage: Bio Bharati Agarose Gel Electrophoresis Teaching kit is stable for 6 months from the date of receipt without showing any change in performance if stored properly as described above.

Important instruction (if any):

- Read the instructions carefully before starting the experiment.
- Dilute the 50 X running buffer to 1X running buffer by adding 49 parts of sterile distilled water* to 1 part of 50X running buffer (e.g. for 500 mL of 1X running buffer add 10 mL of 50 X running buffer to 490 mL of sterile distilled water) and mix well before use.

  *Molecular biology grade water (Cat no: BB-BS60) is recommended for dilution.

Procedure:

1. Prepare the gel tray by sealing the two ends with adhesive tape.
2. Put the comb in the appropriate place of the tray.
3. Prepare the desired amount of 1X running buffer as stated before (in "Important Instruction" section)
4. Weigh the appropriate amount of agarose to make final concentration of 0.7%. (e.g. For 50 mL solution, weigh 0.35 gm of agarose and mix with 50 mL of 1X running buffer)
5. Boil the gel solution until it becomes clear and cool the solution to 50°C to 55 °C.
6. Add 2.5µL of ethidium bromide/50 mL of gel solution provided in the kit and pour into the casting tray and let it solidify (it usually takes around 30-45 minutes in room temperature)
7. To start the run, remove the adhesive tape and place it into the horizontal gel electrophoresis apparatus filled with 1 X running buffer.
8. Carefully remove the comb.
9. Take 5µL of DNA of each sample and mix it with 1 µL of6X loading dye (provided in the kit) and load into the well of the gel.
10. Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black-Cathode. Electrophorese at 80-100 volts (For optimal resolution of DNA greater than 2 kb in size in
11. standard gel electrophoresis, 5 to 8 V/cm is recommended (the distance in cm refers to the distance between electrodes, therefore this recommended voltage would be 5 to 8 multiplied by the distance between the electrodes in cm) until dye markers have migrated an appropriate distance, depending on the size of the DNA to be visualized.
12. Electrophoresis apparatus should always be covered with the lid to avoid electric shocks.
13. Switch off the power supply once the tracking dye from the wells reaches 3/4th of the gel which takes approximately 30-45 minutes.
14. Visualize the gel under UV-Transilluminator with appropriate caution.

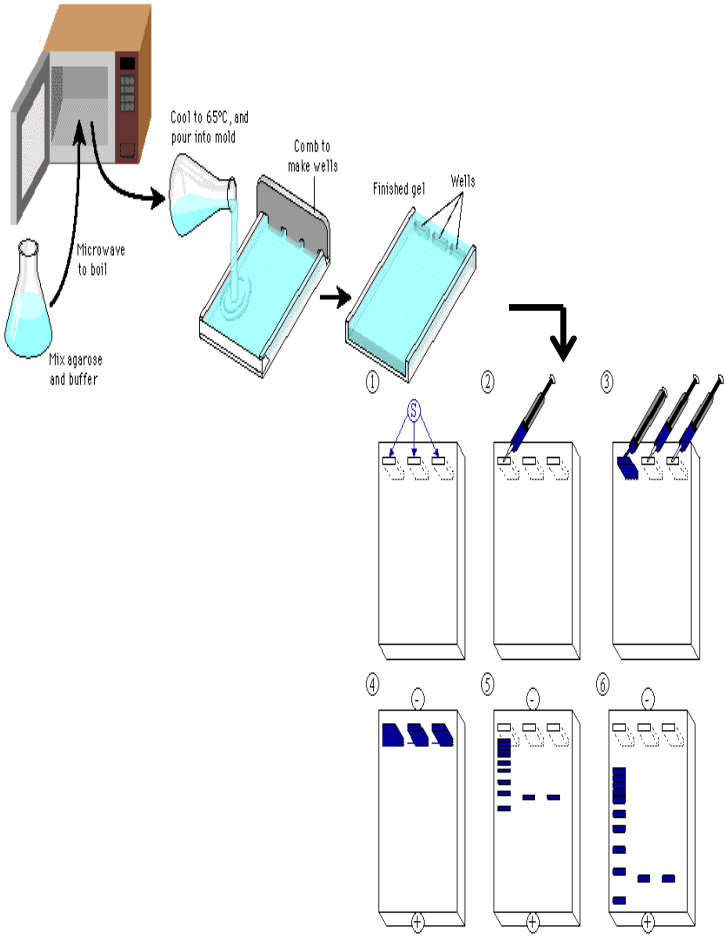

Figure: Flow diagram of Agarose Gel Electrophoresis

Safety:

- **Precautions:**

  UV light is hazardous for skin and eyes. Higher exposure of UV light to skin even lead to cancer. Always wear suitable eye, face and skin protector while working with UV. of UV which also damages DNA. It is advisable to use UV light of low intensity if the DNA is subjected to extraction.

  Ethidium bromide is a mutagenic and moderately toxic substance. So it is strictly advisable to use ethidium bromide with utmost care. Ethidium bromide may present a hazard if it is poured down into the drain without any treatment or placed in the trash. Any ethidium bromide waste should not be poured down the drain, or thrown in the trash, unless the waste has been deactivated or filtered. The following are the recommended disposal procedures for ethidium bromide:

- Electrophoresis Gels, Contaminated Gloves and Other Equipment:
  Treat ethidium bromide gels, contaminated gloves and other equipment which is in contact with ethidium bromide as chemical hazardous waste. There is one form Dry the gels under the hood in an open container before bringing the waste for disposal.
- Sharps Contaminated with Ethidium Bromide:
  Contaminated needles, syringes, etc. must be discarded into a puncture-proof plastic container (must not be red and must not have a biohazard symbol on it) with a lid that closes and must be labelled as ethidium bromide waste.
- Ethidium Bromide Spill Clean-up:
  For small spills (< 20 mL) of concentrations up to 10 mg/mL:
  - Absorb with paper towel /tissue paper and then treat the exposed area with 70-95% ethanol/isopropanol and dispose as hazardous waste.
  - Repeat if necessary (if still fluorescing).
  - Finally rinse with soap and water (paper towels for this step can be discarded into regular trash).
  - DO NOT clean ethidium bromide spills with bleach solutions.
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Interpretation: From agarose gel electrophoresis one can confirm the purity and a rough yield estimation of his/her DNA sample. The DNA loaded into the gel migrates according to their molecular weight. For this reason genomic DNA migrates much slower in comparison with plasmid DNA in the agarose gel as the size of the plasmid DNA is much smaller in comparison with genomic DNA. If the DNA sample is contaminated with RNA that would also be visible as a smear in the lower part of the gel.

Technical Assistance:

At Bio Bharati, we always promise to deliver the best products for quality research to our customers. All the reagents are made at our In-house R&D facility with utmost care to give optimum results. We are always happy to serve you in your research help. For any assistance please write to us at biobharati@gmail.com.

Trouble-shooting Guide:

Sl No	Problem	Reason for Problem	Solution
1.	Faint or no band seen in the gel	DNA electrophoresed off the gel	Reduce the running time. Do not allow the blue tracking dye front of loading dye to run off the gel.
			Check the connection cord. Always connect the cord of the electrophoresis unit as per convention Black: Cathode; Red: Anode.
2.	Smeared DNA bands	DNA was degraded	Ensure the proper storage condition of the given DNA as mentioned in the kit.
		Improper electrophoretic condition	Ensure the gel casting tray is submerged into the buffer.
		Gel electrophoresis buffer reused several times	Discard the buffer and make a fresh one as per instruction
3.	Migration abnomalies	"Smiley" gels	Voltage applied is too high for the gel concentration used. Recalculate the voltage as stated in “Procedure section” and reduce the voltage.
4.	Improper gel solidification	Melting of the gel is not proper	Prepare gel as per the procedure. Ensure that the agarose is dissolved completely.
		Gel kept for too long at room temperature after solidification	Do not let the gel to solidify for prolonged time at room temperature

Adapted from internet