The Biotechnology Education Company®

EDVOTEK®

# 204

EDVO-Kit #

# Separation of RNA & DNA by Gel Filtration Chromatography

**Storage:**
Store entire experiment in the refrigerator.

**Experiment Objective:**

The objective of this experiment is to introduce the principles of gel filtration chromatography as a method to separate RNA and DNA on the basis of size and shape.



All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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## Major Section Headings



## Experiment Components

**Storage:**
Store entire experiment in the refrigerator.

All components of this experiment are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

This experiment is designed for 5 lab groups.

**Contents**

A LyphoSample™
(Lyophilized sample containing RNA, DNA and dyes)
B Dry Matrix
C Concentrated Elution Buffer

10x Gel Loading Solution
Practice Gel Loading Solution
UltraSpec-Agarose™
50x Electrophoresis buffer
DNA Blue InstaStain™
Methylene Blue Plus™
Chromatography columns
1 ml pipet
Transfer pipets
Micro test tubes

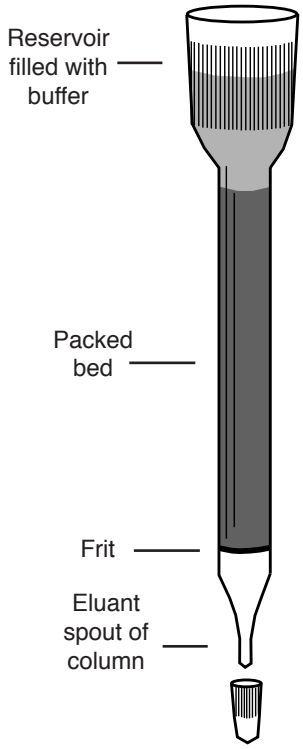
## Requirements

- Horizontal gel electrophoresis apparatus
  (Requires EDVOTEK Model #M12 or equivalent)
- D.C. power supply
- DNA visualization system (white light for methylene blue,
  U.V. transilluminator for ethidium bromide)
- Automatic micropipets with tips
- 6 ring stands with clamps
- 6 - 5 or 10 ml pipets
- 500 - 1000 ml graduated cylinder
- 6 small beakers, flasks, or tubes (10 - 25 ml, for slurry)
- 12 beakers or flasks (50-100 ml, for column eluant and buffer)
- Distilled water

Safety Data Sheets can be found on our website:
**www.edvotek.com/safety-data-sheets**

## Separation of RNA and DNA by Gel Filtration Chromatography

**Quick Reference:**




Gel filtration chromatography (sometimes referred to as molecular sieve chromatography) is a method that separates molecules according to their size and shape. The separation of the components in the sample mixture frequently, but **not** always, correlates with their molecular weights. In these cases, gel filtration can be used as an analytical method to determine the molecular weight of an uncharacterized molecule. Gel filtration is an important preparative technique since it is often a chromatographic step in the purification of proteins, polysaccharides and nucleic acids.

The basic components of the gel filtration experiment are the matrix, chromatography column and the elution buffer. The matrix is the material in the column that actually performs the separation. It is the stationary phase of the chromatography. The column is a tube with a frit and elution spout. The frit is a membrane or porous disk that supports and retains the matrix in the column but allows water and dissolved solutes to pass. The elution buffer is the mobile phase of the chromatography and flows through the matrix and out of the column. The column, with the matrix and applied sample, is "developed" by the elution buffer. This means that the molecules in the sample are carried by the flow of buffer into the matrix where they are gradually separated. The separated zones of molecules then flow out of the column where they are collected for analysis.

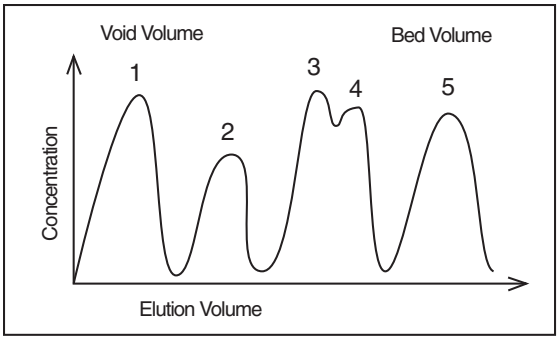
Filling the chromatography column with matrix is called "packing." Matrix that is suspended in the buffer and ready to be poured is referred to as the "slurry." The slurry is carefully poured into the closed column to minimize bubbling and turbulence. A reservoir containing buffer is connected to the column and the column is opened. The flow of buffer forces the matrix down to form an even, homogeneous pack. The packed matrix is called the "bed" and the volume it occupies is termed the "bed volume." It is very important not to allow the bed to run dry. Otherwise, cracks and fissures develop and the matrix has to be removed and repacked.

The gel filtration matrix consists of microscopic beads that contain pores and internal channels. The larger the molecule, the more difficult it is for it to pass through the pores and penetrate the beads. Larger molecules tend to flow around and in between the beads. The total volume of buffer **between** the beads is the "void volume." Smaller molecules tend to spend more time in the maze of channels and pores in the bed. Consequently, the larger, higher molecular weight molecules are eluted from the column **before** smaller molecules. Larger molecules take the faster, more direct path that involves less time in the

# BACKGROUND INFORMATION

**Background Information, continued**

beads. This is somewhat analogous to finding your way out of a complicated maze or simply walking around the outside of the maze and avoiding the whole situation entirely. Molecules can have the same molecular weights but radically different shapes. Molecules with a more compact shape, such as a sphere, will penetrate the beads more easily than those having an elongated shape, like a rod. Therefore, a rodlike molecule will elute before a spherical one of the same molecular weight.




There are many different types of gel filtration matrices. The spectrum of molecular weights the matrix is capable of separating is called the fractionation range. For example, consider a matrix that has a fractionation range (in molecular weight) of 1000 to 100,000. Molecules with an average molecular weight of 1000 or less will not be separated from each other since they all penetrate the beads completely and with equal efficiency. These molecules take the maximum volume of buffer for elution, which is equal to one bed volume. The bed volume is equal to the volume of the beads plus the void volume. Molecules in the range of 1000 to 100,000 will enter the beads with varying efficiencies and be partially or completely separated from one another. Molecules greater than 100,000 will not enter the beads and be eluted in the void volume. Note that in this example, any number of different molecules having molecular weights of 100,000 **or greater** will all elute at the **same** time since they are not sieved by the matrix. The partially or completely separated zones of molecules that are eluted from the column are called peaks. A peak consists of an increasing and decreasing concentration gradient of molecules.

The objective of this experiment is to purify plasmid DNA that is contaminated with RNA by gel filtration chromatography. The plasmid is a circular DNA molecule with a molecular weight of about 2 million. The RNA contaminants consist of degraded fragments of m-RNA, r-RNA and t-RNA with molecular weights in the range of 30,000 to 100,000. The sample also contains two dyes of different molecular weights which will aid in monitoring the chromatography and locating the RNA and DNA. The fractionation range of the matrix is 20,000 to 5 million for nucleic acids (rodlike).

Agarose gel electrophoresis will be used to analyze the RNA and DNA after chromatography. Agarose gel electrophoresis separates nucleic acids on the basis of their size and shape as does gel filtration. However, it possesses greater separation power and is well suited for the analysis of very small quantities of nucleic acids. Unlike the column matrix, the gel does not consist of separate beads. During electrophoresis, the sample molecules must go through the gel.
The agarose gel consists of microscopic pores that act as a molecular sieve. Samples can be loaded into wells made in the gel during casting. Since nucleic acids have a strong negative charge at neutral pH, they

# BACKGROUND INFORMATION

**Background Information, continued**

will migrate through the gel towards the positive electrode in the presence of an electrical field. The rate of migration is affected by the pores in the gel. Smaller molecules pass through the pores more easily and will migrate ahead of the plasmid DNA. The same plasmid molecules can form interlocking rings with themselves during replication in the cell. These multimers of plasmids are called catenanes. Catenanes consisting of two rings are "dimers" and three rings are "trimers." Higher catenanes also occur. It is difficult to separate catenanes from their single circle counterparts with the type of gel filtration you will be doing in this laboratory. In contrast, agarose gel electrophoresis easily separates catenanes. Single circles migrate faster than dimers, dimers faster than trimers, etc.

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# EXPERIMENTAL PROCEDURES


## EXPERIMENT OBJECTIVE:

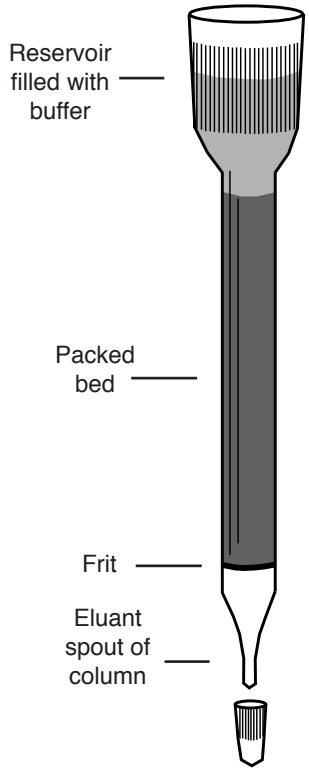
The objective of this experiment is to introduce the principles of gel filtration chromatography as a method to separate RNA and DNA on the basis of size and shape.

## LABORATORY SAFETY

Gloves and goggles should be worn routinely as good laboratory practice.

## Chromatography: Packing the Column

**Quick Reference:**



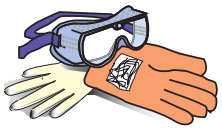

1. Mix the matrix thoroughly by swirling or gently stirring.
2. With a 5 or 10 ml pipet, carefully pipet all of the mixed slurry into the column by letting it stream down the inside walls of the reservoir funnel.

   If the flow of matrix is stopped by an air pocket, stop pouring and firmly tap the column until the air is removed and the slurry flows down. Continue pouring the rest of the slurry.
3. With a 5 or 10 ml pipet, add approximately 5 ml of elution buffer to the reservoir.
4. Place an empty beaker under the column.
5. Remove the cap from the spout of the column.
6. Let the buffer flow through the column for approximately 10 minutes. The matrix will pack down into the column.
7. Place the cap onto the spout of the column.
8. The matrix is packed when it stops compressing. The volume of the packed bed will be approximately 3 ml.

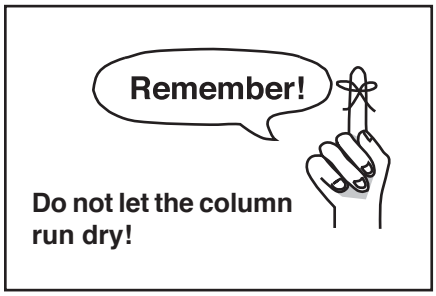

## OPTIONAL STOPPING POINT #1

If time is limited, the experiment can be temporarily stopped here. **The columns must be securely closed so they do not run dry.** If you are stopping the experiment here, make sure there is buffer above the bed and the reservoir is covered with plastic wrap or parafilm.

## Chromatography: Fraction Collection



Wear gloves and safety goggles




1. Label 6 test tubes 1-6. Put your initials or lab group number on all the tubes.
2. Carefully remove all the buffer from above the bed with a transfer pipet. The top of the bed should be exposed to air.

   Insert the pipet through the reservoir. Try to minimize disturbance of the bed while removing buffer.
3. Load the contents of the "Sample" tube onto the top of the bed with a transfer pipet. Let the sample drip down the inside walls of the column.
4. Place a beaker under the column.
5. Remove the cap from the spout. The sample will slowly enter the bed. When it has **completely** entered the bed (the top of the bed will be exposed to air), replace the cap.
6. **Carefully** add buffer over the bed with a transfer pipet several drops at a time. Continue adding buffer until the reservoir is almost full.
7. Hold test tube #1 directly under the column. Remove the cap from the spout.
8. Using the microtest tube containing 0.5 ml of water as your guide, collect 0.5 ml of column effluent in each of the tubes 1-6.
9. As the dyes gradually separate in the column, periodically add fresh buffer to the reservoir to keep it full.
10. After all six tubes of column effluent (column fractions) have been collected, replace the cap onto the spout.


**Optional Stopping Point #2**

The experiment may be stopped after the column fractions are collected. The fractions may be stored frozen until they are needed for electrophoresis.

# EXPERIMENTAL PROCEDURES

## Preparing Samples for Electrophoresis

### OPTION ONE:

Prepare all column fractions for electrophoresis

1. Label 6 microtest tubes 1-6.
2. With a micropipet, transfer 50 µl from each column fraction to its corresponding new sample tube.

   **Use a fresh micropipet tip for each transfer**.
3. Add 5 µl of 10x gel loading solution to each 50 µl aliquot. Mix by tapping the tube.

   You do not need to use a fresh micropipet tip for each addition of gel loading solution to the sample tubes.
4. All six (6) samples are now ready for electrophoresis.


The peak of the blue dye coelutes with plasmid, while the peak of orange dye coelutes with RNA.

### OPTION TWO:

Prepare the peak column fractions for electrophoresis

1. Label 2 microtest tubes X and Y.
2. Identify the column fraction with the greatest amount of blue dye.

   Holding the collection tubes against a white background or on a white light box may help to identify the peaks.
3. With a micopipet, transfer 50 µl into Tube X.
4. Identify the column fraction that has the greatest amount of orange dye.
5. With a fresh micropipet tip, transfer 50 µl into Tube Y.

*continued*

**Preparing Samples for Electrophoresis, continued**

6. Add 5 µl of 10x gel loading solution to each of tubes X and Y. Mix by shaking the tube.

   You do not need to use a fresh micropipet tip for each addition of gel loading solution to the sample tubes.

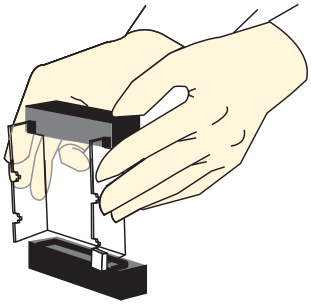
7. The samples are now ready for electrophoresis.


### OPTIONAL STOPPING POINT #3

The experiment may be stopped after the samples are prepared. Samples may be stored in the freezer until they are needed for electrophoresis. If samples will be stored before electrophoresis, initial your tubes or put your lab group # on them.

## Agarose Gel Preparation

### PREPARING THE GEL BED



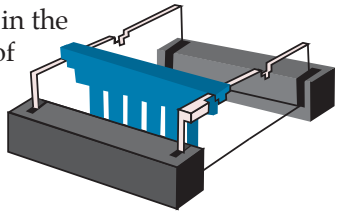
**Using 7 x 7 cm Gel Beds**

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.

   A. Using Rubber dams:
   - Place a rubber dam on each end of the bed. Make sure the rubber dam sits firmly in contact with the sides and bottom of the bed.

   B. Taping with labeling or masking tape:
   - With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
   - Fold the extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.

2. Place a well-former template (comb) in the first set of notches nearest the end of the gel bed. Make sure the comb sits firmly and evenly across the bed.



# EXPERIMENTAL PROCEDURES

**Agarose Gel Preparation, continued**

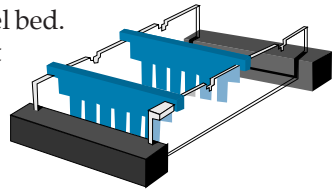
## Using the 7 x 15 cm Gel Bed for Two Gels

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.

   A. Using Rubber dams:
   - Place a rubber dam on each end of the bed. Make sure the rubber dam sits firmly in contact with the sides and bottom of the bed.

   B. Taping with labeling or masking tape:
   - With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
   - Fold the extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.

2. Place a well-former template (comb) in the first set of notches nearest the end of the gel bed. Place a second comb in the middle set of notches. Make sure the combs sit firmly and evenly across the bed.



Wear gloves and safety goggles

## CASTING THE GEL

This experiment requires a 0.8% gel.

3. Use a 250 ml flask to prepare the diluted gel buffer.

**Table A: Individual 0.8 % UltraSpec-Agarose™ Gels for staining with DNA Blue InstaStain™**

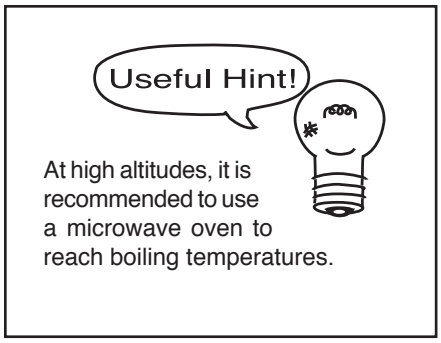
| Size of EDVOTEK Casting Tray | Amt of Agarose | + | Concentrated Buffer (50x) | + | Distilled Water | = | Total Volume |
|---|---|---|---|---|---|---|---|
| 7 x 7 cm | 0.24 gm | | 0.6 ml | | 29.4 ml | | 30 ml |
| 7 x 15 cm | 0.48 gm | | 1.2 ml | | 58.8 ml | | 60 ml |
| 10.5 x 14 cm | 0.8 gm | | 2.0 ml | | 98.0 ml | | 100 ml |

- With a 1 ml pipet, measure the buffer concentrate and add the distilled water as indicated in **Table A.**

4. Add the required amount of agarose powder. Swirl to disperse clumps.

# EXPERIMENTAL PROCEDURES

## Agarose Gel Preparation, continued



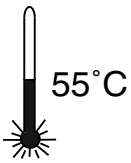

5. With a marking pen, indicate the level of the solution volume on the outside of the flask.

6. Heat the mixture to dissolve the agarose powder. The final solution should be clear (like water) without any undissolved particles.

   A. Microwave method:
   - Cover flask with plastic wrap to minimize evaporation.
   - Heat the mixture on High for 1 minute.
   - Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.

   B. Hot plate or burner method:
   - Cover the flask with foil to prevent excess evaporation.
   - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

7. Cool the agarose solution to 55°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 5.





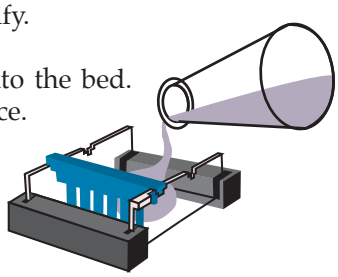
**DO NOT POUR BOILING HOT AGAROSE INTO THE GEL BED.**
Hot agarose solution may irreversibly warp the bed.

**After the gel is cooled to 55°C:**

If using rubber dams, go to step 9. If using tape, continue with step 8.

8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.
   - Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
   - Wait approximately 1 minute for the agarose to solidify.

9. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.



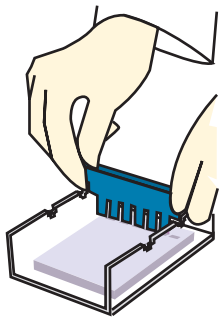
10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

**Agarose Gel Preparation, continued**


Step 11: Be careful not to damage or tear the gel when removing rubber dams. A thin plastic knife or spatula can be inserted between the gel and the dams to break possible surface tension.

## PREPARING THE GEL FOR ELECTROPHORESIS



11. After the gel is completely solidified, carefully and slowly remove the rubber dams or tape.

12. Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.

13. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.

14. Fill the electrophoresis apparatus chamber with the required volume of diluted buffer (see guidelines presented in Table B).

**Table B: Electrophoresis (Chamber) Buffer**

| EDVOTEK Model # | Concentrated Buffer (50x) | + | Distilled Water | = | Total Volume |
|---|---|---|---|---|---|
| M6 | 4 ml | | 196 ml | | 200 ml |
| M6 + | 6 ml | | 294 ml | | 300 ml |
| M12, M20 | 8 ml | | 392 ml | | 400 ml |
| M36 | 10 ml | | 490 ml | | 500 ml |

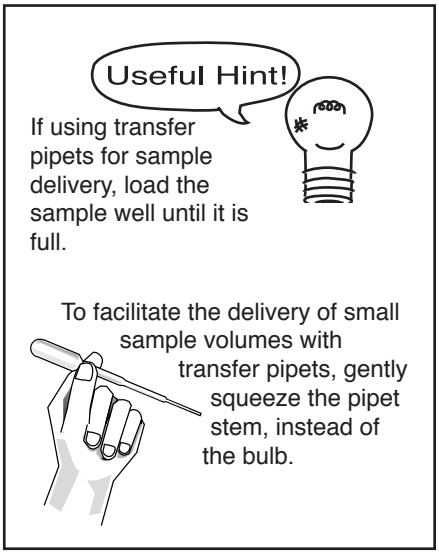
15. Make sure the gel is completely covered with buffer. The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.

16. Load samples in wells and conduct electrophoresis according to experiment instructions starting on page 15.

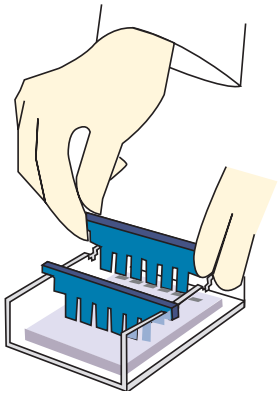
## Sample Delivery and Practice Gel Loading

An automatic micropipet is used to deliver accurate, reproducible volumes of sample. For gels to be stained with Methylene Blue Plus™ or DNA Blue InstaStain™, load the sample well with 35 - 38 microliters of sample. Check with your instructor regarding the amount of sample you should be delivering.

With the EDVOTEK system, an alternative sample delivery method with disposable microtipped transfer pipets can be used. Transfer pipets are not precise, and because their volumes can not be accurately controlled, significant sample waste can occur. Delivery of small sample volumes with transfer pipets can be facilitated by gently squeezing the pipet stem, instead of the bulb.




### PRACTICE GEL LOADING



If you are unfamiliar with loading samples in agarose gels, it is recommended that you practice sample delivery techniques before conducting the experiment. EDVOTEK electrophoresis experiments contain a tube of practice gel loading solution. Casting of a separate practice gel is highly recommended. One suggested activity is outlined below:

1. Cast a gel with the maximum number of wells and place it under buffer in an electrophoresis apparatus chamber.

   Alternatively, your teacher may have cut the gel in sections between the rows of wells. Place a gel section with wells into a small, shallow tray and submerge under water.

2. Practice delivering the practice solution to the sample wells. Take care not to damage or puncture the wells with the pipet tip.

3. If you need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.

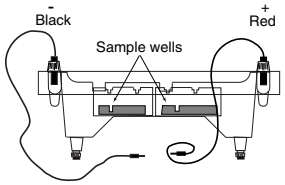
4. Replace the practice gel with a fresh gel for the actual experiment. The practice gel loading solution is diluted in the buffer and will not interfere with the experiment.

# EXPERIMENTAL PROCEDURES

## Agarose Gel Electrophoresis

**Reminder:**

During electrophoresis, the DNA samples migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.




**Quick Reference:**

If you are using an automatic micropipet to deliver samples, the amount of sample that should be loaded is 35-38 µl.

**Table C: Time and Voltage**

| Volts | Recommended Time | |
|---|---|---|
| | Minimum | Optimal |
| 50 | 60 min | 2.0 hrs |
| 70 | 40 min | 1.5 hrs |
| 125 | 30 min | 45 min |

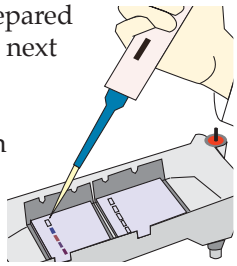
* The EDVOTEK Model #M6 should not be run at higher than 70 volts.

A 0.8% agarose gel is required for the experiment.

### LOADING ALL DNA SAMPLES (OPTION 1)

1. Load each of the prepared samples into the wells in consecutive order.

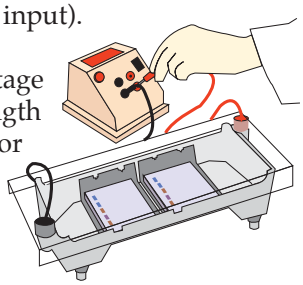
### LOADING THE PEAK DNA SAMPLES (OPTION 2)



1. Each lab group should consecutively load prepared samples from tubes X and Y in separate wells next to each other.
2. Remember to note the well numbers in which your group loaded samples.

### RUNNING THE GEL

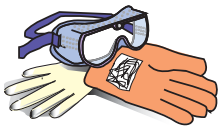
1. After the samples are loaded, carefully snap the cover down onto the electrode terminals. Make sure that the negative and positive indicators on the cover and apparatus chamber are properly oriented.
2. Insert the plug of the black wire into the black input of the power supply (negative input). Insert the plug of the red wire into the red input of the power supply (positive input).



3. Set the power supply at the required voltage and run the electrophoresis for the length of time as determined by your instructor When current is flowing properly, you should see bubbles forming on the electrodes.
4. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.
5. Remove the gel on its bed. Place your hands on each end of the gel to prevent it from slipping off the bed.
6. Transfer the gel for staining and visualization with DNA Blue InstaStain™ or Methylene Blue Plus™.

## Staining & Visualization of DNA

**NEW**
**Stain DNA with DNA Blue InstaStain™**
**Patents Pending**



Wear gloves and safety goggles

### DNA BLUE INSTASTAIN™

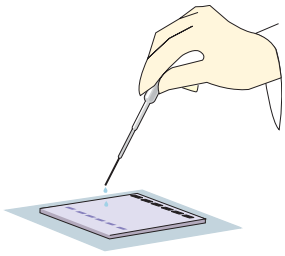
EDVOTEK Series 100 electrophoresis experiments now feature a new proprietary staining method for staining DNA separated on agarose gels. Based on state-of-the-art technology, DNA Blue InstaStain™ is safe, quick, and minimizes the mess of conventional DNA staining with blue stains.

**Advantages of DNA Blue InstaStain™ vs. Liquid Staining**

- Safe and Simple to Use
- Quick 15-minute staining
- Uniformity of Staining
- Minimal liquid waste

### Staining with DNA Blue InstaStain™

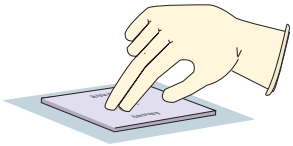
1. After electrophoresis is completed, place the gel on a flat surface. Moisten the gel with several drops of electrophoresis buffer.



2. Wearing gloves, place the blue side of the DNA Blue InstaStain sheet on the well-moistened gel.

3. Firmly run your fingers over the entire surface of the DNA InstaStain.

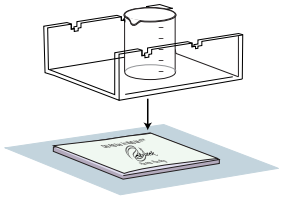
   Do this several times.



4. Place the gel and DNA Blue InstaStain on a piece of plastic wrap. Then put the gel casting tray and a small empty beaker on top.

   *This will ensure that the InstaStain sheet maintains good contact with the gel surface.*

   Allow the DNA Blue InstaStain™ to sit for 15 minutes.



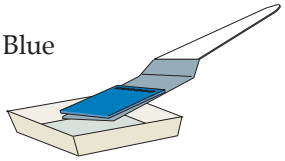
 

# EXPERIMENTAL PROCEDURES

## Staining & Visualization of DNA, cont.

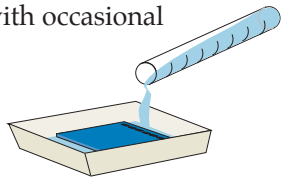
### Destaining and Visualization of DNA

5. After 15 minutes, remove the sheet of DNA Blue InstaStain and transfer the gel to a large weigh boat or small plastic container.



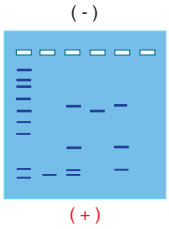
6. Conduct destaining with distilled water that has been warmed to 37°C.

   - First destain: submerge the gel under a small amount of 37°C distilled water for 10 minutes with occasional agitation.
   - Second and third destain: submerge the gel under a small amount of 37°C distilled water for another 10 minutes with occasional agitation.



DO NOT EXCEED 37°C ! Warmer temperatures will soften the gel and may cause it to break.

7. After the first destain, the larger DNA bands will be visible as dark blue bands against a lighter blue background. When completely destained, the dark blue DNA bands will become clearer and the entire background will become uniformly light blue in color.




8. Carefully remove the gel from the destain solution and examine the gel on a Visible Light Gel Visualization System. To optimize visibility, use the amber filter provided with EDVOTEK equipment.

9. If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.

### Storage and Disposal of Gel

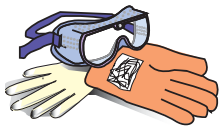
- A gel stained with DNA Blue InstaStain™ may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.

DO NOT FREEZE AGAROSE GELS.

- Stained gels which are not kept can be discarded in solid waste disposal.


A large weigh boat works well for destaining a 7 x 7 cm gel. Use 50 ml of 37°C distilled water to submerge the gel in a large weigh boat.

**Staining & Visualization of DNA, cont.**



Wear gloves and safety goggles

## STAINING AND DESTAINING 5 GELS SIMULTANEOUSLY WITH METHYLENE BLUE PLUS™

1. Remove each gel from its bed and totally submerse 5 gels together in one tray containing 600 ml of diluted Methylene Blue Plus™ stain.

   Do not stain gels in the electrophoresis apparatus.

2. Stain gels for a minimum of 30 minutes, with occasional stirring.

3. Conduct destaining twice in 600 ml of distilled water that has been warmed to 37°C.

   - First destain: completely submerse the gels in 600 ml of 37°C distilled water for 15 minutes. Then discard the destaining solution
   - Second and third destain: completely submerse the gels in 600 ml of 37°C distilled water for another 15 minutes.

   Bands will start to become clearly visible after the second destain. You may also leave the gels in destain overnight.

DO NOT EXCEED 37°C ! Warmer temperatures will soften the gel and may cause it to break.

5. Carefully remove the gel from the destain solution and examine the gel on a Visible Light Gel Visualization System. To optimize visibility, use the amber filter provided with EDVOTEK equipment.

6. If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.

### Storage and Disposal of Gel

- A gel stained with Methylene Blue Plus™ may be stored in the refrigerator for several weeks. Place the gel in a Gel-Save Cassette or sealable plastic bag with destaining liquid.

  DO NOT FREEZE AGAROSE GELS.

- Stained gels which are not kept can be discarded in the trash.


A gel that has been electrophoresed can remain overnight in the apparatus under buffer and stained the next day. For optimal results, stain the gel immediately after terminating the electrophoresis.

After electrophoresis, each group should remove a small slice, or make a small hole in a designated corner of their gel, to facilitate identification after staining and destaining.

## Study Questions

1. Why did most of the blue dye coelute with the plasmid DNA? Why did the RNA elute after the DNA?

2. Explain two ways that would improve the separation of the RNA and DNA.

3. The matrix used in the experiment has a fractionation range of 20,000 to 5 x $10^6$. Would you use the matrix to purify a plasmid that had a molecular weight of 6 x $10^6$ from partially broken chromosomal DNA with an average fragment molecular weight of 1.5 x $10^6$?

4. How is the separation of degraded RNA and plasmid DNA different during agarose gel electrophoresis when compared to gel filtration chromatography? Why?

5. A sample containing three proteins having molecular weights of 70,000, 466,000 and 468,000 were submitted to gel filtration chromatography. Two peaks were expected in the elution profile one containing to 70,000 Dalton protein and the other containing both large proteins. However, three peaks were found. The earliest two peaks were eluted relatively close to each other but were resolved. The 466,000 Dalton protein was eluted first. Gel filtration matrices cannot usually distinguish large proteins that differ by only 2,000 Daltons. Explain the results.

## Notes

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# INSTRUCTOR'S GUIDE

## General Information


A solidified gel can be stored in the covered electrophoresis apparatus, **under** buffer in the refrigerator for up to 2 weeks.

If samples are loaded, the gel must be electrophoresed right away because the samples will diffuse out of the wells.

### HOW THIS EXPERIMENT IS ORGANIZED:

This experiment module is designed for five groups and consists of two major parts: 1) Chromatography, which is followed by 2) agarose gel electrophoresis. Two procedural options for conducting the electrophoresis are presented in the Experimental Procedures.

1. Procedure Option 1 should be performed if students will conduct electrophoresis of all samples prepared from column fractions collected from the chromatography.

2. Procedure Option 2 should be performed if only the peak column fractions from each lab group will be prepared and pooled for analysis on one agarose gel.

If you have five electrophoresis units, one for each of the lab groups, electrophoresis can be performed simultaneously by all five groups. Alternatively, various lab groups can store their samples and perform the electrophoresis at different times (see Optional Stopping Points 2 and 3, in the Experimental Procedures, page 8 and 10 respectively).

### APPROXIMATE TIME REQUIREMENTS FOR PRE-LAB AND EXPERIMENTAL PROCEDURES

1. The dry matrix must be swelled 1 to 24 hours before the lab. Pre-lab preparation of column chromatography reagents takes approximately one hour.

2. Agarose gel preparation: Your schedule will determine when to prepare the agarose gel(s). Whether you choose to prepare the gel(s), or have the students do it, allow approximately 30 to 40 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification.

3. Students will require approximately 10 minutes to pack the column and 20 minutes to collect fractions.

## Pre--Lab Preparations

Wear gloves and safety goggles

**Quick Reference:**

The sample mixture contains RNA, DNA and dyes. Gel filtration is used to remove the majority of degraded, contaminating RNA from plasmid DNA. The separation of two nucleic acids (and the added dyes) is done on the basis of size and shape Larger molecules elute first.

### PREPARATION OF COLUMNS

1. Vertically mount the column on a ring stand, making sure it is straight.
2. Slide the column cap onto the spout at the bottom of the column.

### PREPARATION OF ELUTION BUFFER

1. To 360 ml of distilled water, pour all of the concentrated elution buffer (C). Mix.
2. Dispense 50 ml of diluted buffer in a beaker for each lab group. Keep the extra buffer on hand in case of spills during the lab.
3. Pipet 0.5 ml of water into each of 5 microtest tubes. The level of water in the tube will be used as a reference guide for the equal collection of column effluent.
4. Before the column is packed, add 0.2 ml of buffer to each empty column. Make sure the buffer is at the bottom of the column.

### PREPARATION OF MATRIX

**(One hour before the lab or overnight)**

1. Add the dry matrix (B) to a tube or beaker. Add 26 ml diluted elution buffer.
2. Cover the tube or beaker and swirl to mix the matrix.
3. Allow the matrix to swell for at least 1 hour at room temperature before dispensing.
4. After the matrix has swollen, stir or swirl to make a uniform suspension and quickly dispense 5 ml into a small beaker, flask, or tube for each group.

**Pre-Lab Preparations, continued**

## PREPARATION OF SAMPLE MIXTURE

On the day of the Chromatography Experiment:

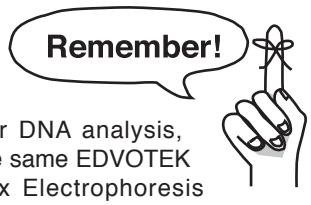
1. Add 0.6 ml of distilled water to the LyphoSample (A.). Cap the vial and mix until all the freeze-dried material is dissolved.

2. Dispense 0.12 ml to each of five tubes labeled "Sample". Store frozen if not used immediately.

**Each lab group should receive:**

| | | |
|---|---|---|
| 1 | column attached to a ring stand | |
| 12 | microtest tubes | |
| 2 | transfer pipets | |
| 1 | empty beaker | |
| 1 | 5 or 10 ml pipet | |
| 1 | pipet pump or bulb | |
| 1 | beaker with matrix | 5.0 ml |
| 1 | beaker of elution buffer | 50.0 ml |
| 1 | microtest tube of sample | 0.12 ml |
| 1 | microtest tube of water | 0.5 ml |

**Pre-Lab Preparations, continued**



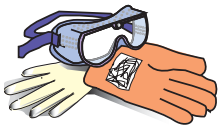

For DNA analysis, the same EDVOTEK 50x Electrophoresis Buffer is used for preparing both the agarose gel buffer and the chamber buffer. To dilute: add 1 volume of buffer concentrate to every 49 volumes of distilled or deionized water. Do not use tap water.

## ELECTROPHORESIS BUFFER

The electrophoresis (chamber) buffer recommended is Tris-acetate-EDTA (20 mM tris, 6 mM sodium acetate, 1 mM disodium ethylenediamine tetraacetic acid) pH 7.8. Prepare the buffer as required for your electrophoresis apparatus as outlined in Table B below. (Table B also appears previously on page 13). To dilute EDVOTEK (50x) concentrated buffer, add 1 volume of buffer concentrate to every 49 volumes of distilled or deionized water.

**Table B: Electrophoresis (Chamber) Buffer**

| EDVOTEK Model # | Concentrated Buffer (50x) | + Distilled Water | = Total Volume |
|---|---|---|---|
| M6 | 4 ml | 196 ml | 200 ml |
| M6 + | 6 ml | 294 ml | 300 ml |
| M12, M20 | 8 ml | 392 ml | 400 ml |
| M36 | 10 ml | 490 ml | 500 ml |



Wear gloves and safety goggles

## STAINING, STORAGE AND DISPOSAL OF GELS

### When Using DNA Blue InstaStain™

A gel stained with DNA Blue InstaStain™ may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with several drops of destaining liquid. DO NOT FREEZE AGAROSE GELS.

Used InstaStain™ sheets and destained gels which are not kept can be discarded in the trash. Destaining solutions can be disposed down the drain.

**Dilution of Methylene Blue Plus™ stain:**

Dilute the 10x stain by mixing 1 part stain with 9 parts distilled or deionized water.

### When Using Methylene Blue Plus™ Stain

A gel stained with Methylene Blue Plus™ may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid. DO NOT FREEZE AGAROSE GELS.

Stained gels which are not kept can be discarded in the trash. Destaining solutions can be disposed down the drain.

# Electrophoresis Hints and Help

## NOTES REGARDING ELECTROPHORESIS

1. Do not move the apparatus immediately after the samples have been loaded.
   - Moving the apparatus will dislodge the samples from the wells into the buffer and will compromise results.
   - If it is necessary to move the apparatus during electrophoresis, you may safely do so after the tracking dye has migrated at least 1 cm from the wells into the gel.

2. For optimal DNA fragment separation, do not use voltages higher than 125 volts for agarose gel electrophoresis. Higher voltages can overheat and melt the gel.

3. The DNA samples contain tracking dye, which moves through the gel ahead of most DNA (except extremely small fragments). Migration of the tracking dye will become clearly visible in the gel after approximately 10-15 minutes.

4. If DNA fragments are similar in size, fragments will migrate close to one another.
   - In general, longer electrophoretic runs will increase the separation between fragments of similar size.
   - Experiments which involve measurement of fragment molecular size or weight should be run at the recommended optimal time to ensure adequate separation.

5. Electrophoresis should be terminated when the tracking dye has moved 3.5 to 4 centimeters from the wells and before it moves off the gel.
   - For optimal results, stain the gel immediately after electrophoresis.
   - The gel can remain overnight in the apparatus under buffer and stained the next day.
   - For convenience, the power source can be connected to a household automatic light timer to avoid running samples off the end of the gel.

## CARE AND MAINTENANCE OF THE ELECTROPHORESIS APPARATUS

1. The temperature of the melted agarose which is poured into the bed during gel casting should not exceed 55°C. Hot agarose solution may irreversibly warp the casting tray.

**Electrophoresis Hints and Help continued**

2. Avoid touching the fragile platinum electrodes.

3. Power should always be turned off and leads disconnected from the power source when the cover is removed from the apparatus.

4. To clean the apparatus chamber, gel casting tray and combs, rinse well with tap water. If your tap water is high in minerals, give the items a final rinse with distilled water. Let air dry. Do not use detergents of any kind, or expose the apparatus to alcohols.

5. Should an unlikely leak develop in any electrophoresis apparatus you are using, IMMEDIATELY SHUT OFF POWER. Do not use the apparatus. Call 1-800-EDVOTEK.

## AVOIDING COMMON PITFALLS

Potential pitfalls and/or problems can be avoided by following the suggestions and reminders listed below.

- When packing columns, avoid bubbles and air pockets that will interrupt the flow of the sample.

- To ensure that DNA bands are well resolved, make sure the gel formulation is correct (see Table A) and that electrophoresis is conducted for the optimal recommended amount of time.

- Correctly dilute the concentrated buffer for preparation of both the gel and electrophoresis (chamber) buffer. Remember that without buffer in the gel, there will be no DNA mobility. Use only distilled water to prepare buffers. Do not use tap water.

- For optimal results, use fresh electrophoresis buffer and Methylene Blue Plus™ stain prepared according to instructions.

- Before performing the actual experiment, practice sample delivery techniques to avoid diluting the sample with buffer during gel loading.

- To avoid loss of DNA fragments into the buffer, make sure the gel is properly oriented so the samples are not electrophoresed in the wrong direction off the gel.

- If DNA bands appear faint after staining and destaining, repeat the procedure. Although 30 minutes is sufficient, staining for a longer period of time will not harm the gel.

**Please refer to the kit insert for the Answers to Study Questions**