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Edvo-Kit # 204

Edvo-Kit #204

Separation of RNA & DNA by Gel Filtration Chromatography

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.

See page 3 for storage instructions.

Version 204.220602

SAMPLE LITERATURE
Please refer to included weblink for correct version

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

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Experiment Components

Components	Check (✓)
Store components A-C in the refrigerator.	
A LyphoSample™ (Lyophilized sample containing RNA, DNA and dyes)	❑
B Dry Matrix	❑
C Concentrated Elution Buffer	❑

Experiment #204 is designed for 5 groups.

REAGENTS & SUPPLIES

Store the following at room temperature.

- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer (50x) ❑
- 10x Gel Loading Solution ❑
- Practice Gel Loading Solution ❑
- FlashBlue™ DNA Stain ❑
- Chromatography columns ❑
- 1 mL pipet ❑
- Transfer pipets ❑
- Micro test tubes ❑

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipettes with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 mL flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel destaining)
- DNA visualization system (white light)
- Distilled water
- 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)

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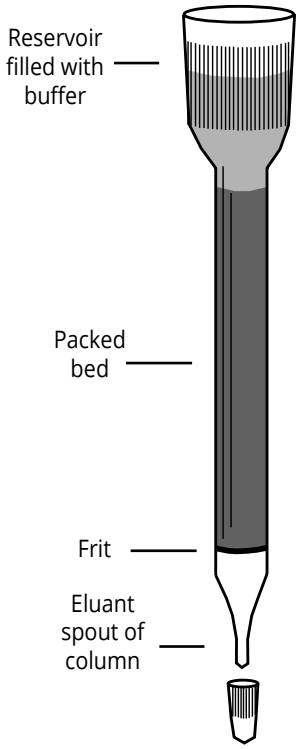
Background Information

SEPARATION OF RNA AND DNA BY GEL FILTRATION CHROMATOGRAPHY

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.

Quick Reference:



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
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

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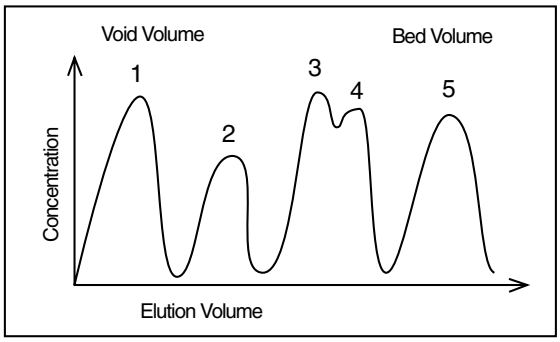
elution, which is equal to one bed volume. The bed volume is equal to the volume of the beads plus the void volume. Molecules in the range of 1000 to 100,000 will enter the beads with varying efficiencies and be partially or completely separated from one another. Molecules greater than 100,000 will not 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 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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Experiment Overview

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

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

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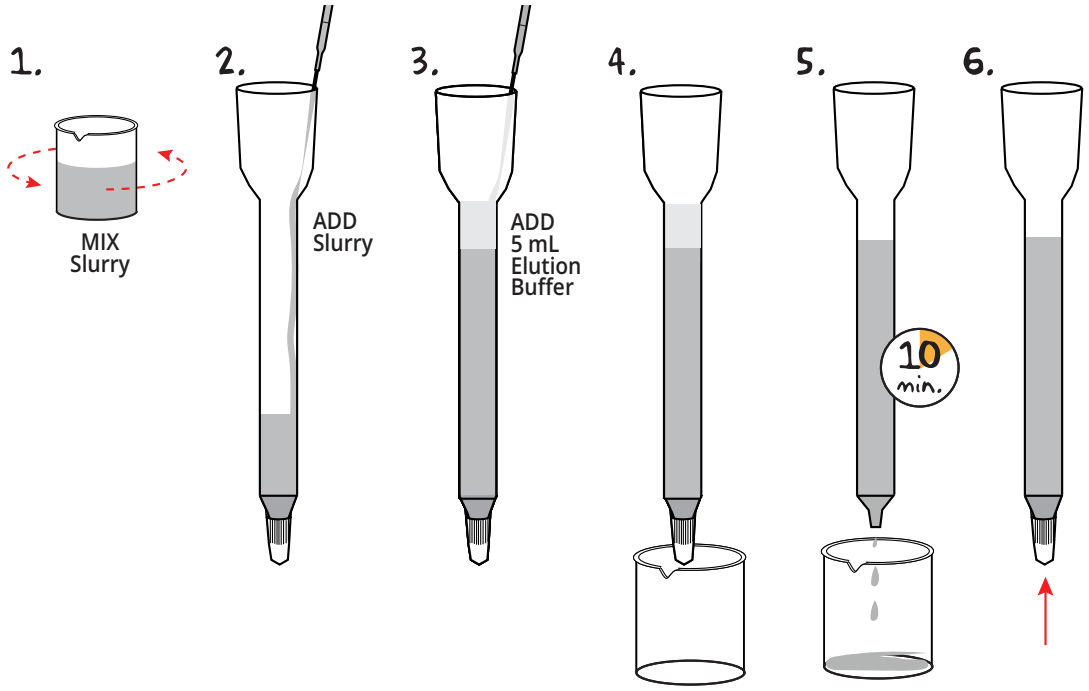
Chromatography: Packing the Column



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 and let the buffer **FLOW** through the column for approximately 10 minutes. The matrix will pack down into the column.
6. **PLACE** the cap onto the spout of the column. 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.

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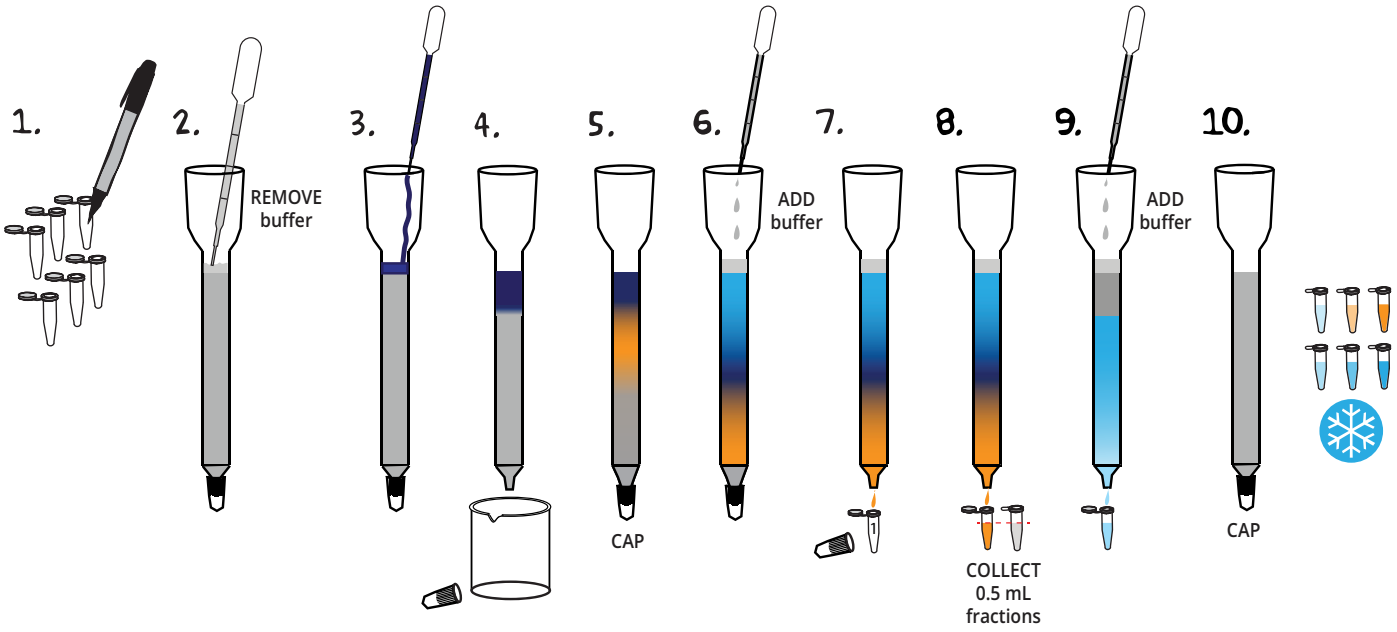
Chromatography: Fraction Collection



1. **LABEL** six 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. Try to minimize disturbance of the bed while removing buffer. The top of the bed should be exposed to air.
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 and **REMOVE** the cap from the spout. The sample will slowly enter the bed.
5. 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. ***DO NOT ALLOW THE COLUMN TO GO DRY!***
10. After all six tubes of column effluent (column fractions) have been collected, **REPLACE** the cap onto the spout.

Wear gloves and safety goggles

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.

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Chromatography: Preparing Samples for Electrophoresis

OPTION ONE: Prepare all column fractions for electrophoresis.

1. **LABEL** six microtest tubes 1-6.
2. With a micropipette, **TRANSFER** 50 µL from each column fraction to its corresponding new sample tube. ***NOTE: Use a fresh micropipette tip for each transfer.***
3. **ADD** 5 µL of 10x gel loading solution to each of the tubes 1-6. **MIX** by tapping the tube. You do not need to use a fresh micropipette tip for each addition of gel loading solution to the sample tubes.
4. The samples are now ready for electrophoresis.

OPTION TWO: Prepare the peak column fractions for electrophoresis.

1. **LABEL** two 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 micopipette, **TRANSFER** 50 µL into Tube X.
4. **IDENTIFY** the column fraction that has the greatest amount of orange dye.
5. With a fresh micropipette tip, **TRANSFER** 50 µL into Tube Y.
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 micropipette tip for each addition of gel loading solution to the sample tubes.
7. The samples are now ready for electrophoresis.

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

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.

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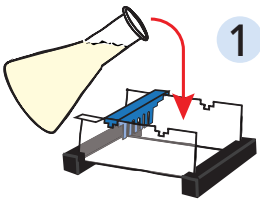
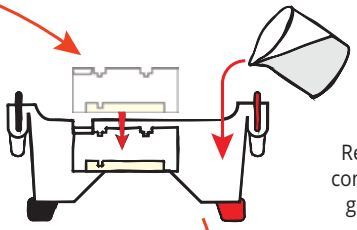
Electrophoresis Overview

Agarose Gel Electrophoresis

Time required: See Table C



1 Prepare agarose gel in casting tray.



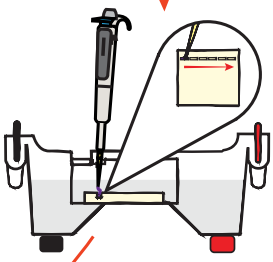
2 Remove end caps & comb, then submerge gel under buffer in electrophoresis chamber.

Option One - Running all six DNA samples

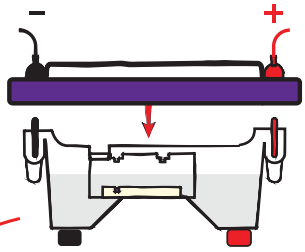
Load each of the 6 prepared samples into the wells in consecutive order.

Option Two - Running the peak DNA samples

Load prepared samples from tubes X and Y in separate wells next to each other.



3 Load each sample in consecutive wells

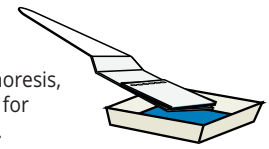


4 Attach safety cover & connect leads to power source to conduct electrophoresis.

Staining Agarose Gels Using FlashBlue™

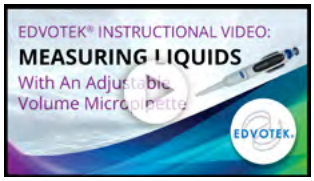
Time required: 30 min.

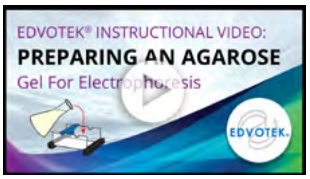
5 After electrophoresis, transfer gel for staining.



6 Analysis on white light source.

Related EDVOTEK® Instructional Videos





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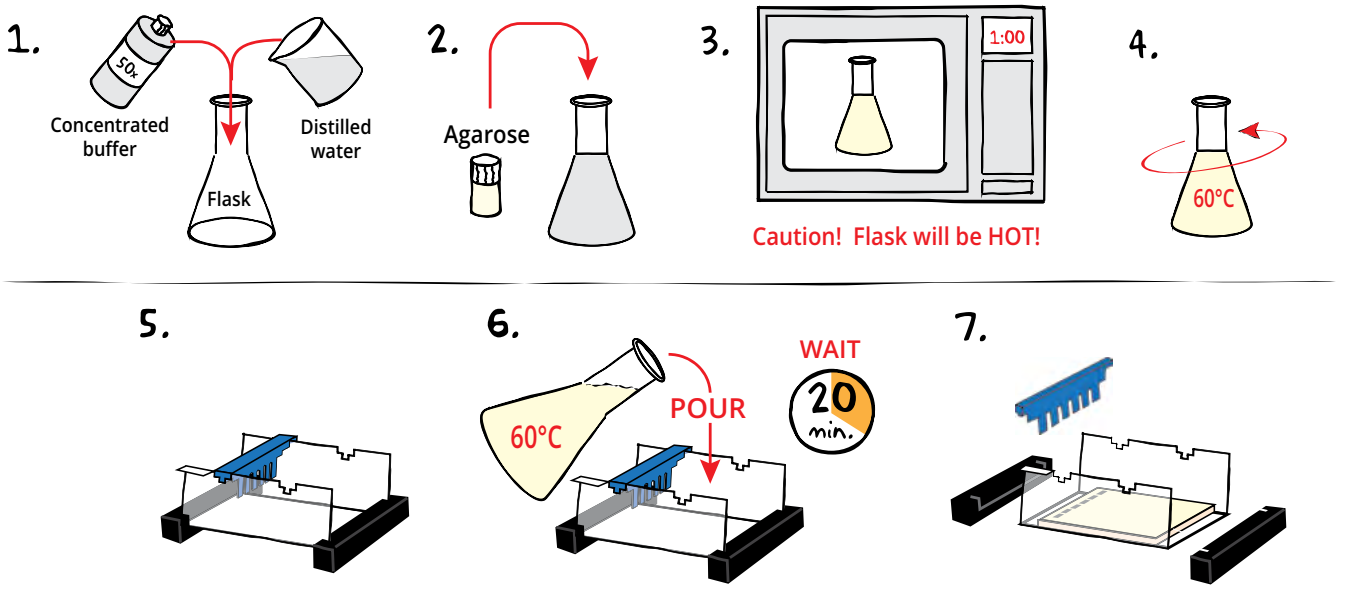
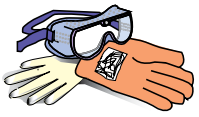
Agarose Gel Electrophoresis



CASTING THE AGAROSE GEL

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.



Wear gloves and safety goggles

REMINDER:
This experiment requires 0.8% agarose gels cast with 6 wells.

Table A — **Individual 0.8% UltraSpec-Agarose™ Gels**

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 mL	29.4 mL	0.24 g	30 mL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL

**Recommended gel volume for the EDGE™ Integrated Electrophoresis System. (Cat. #500).*

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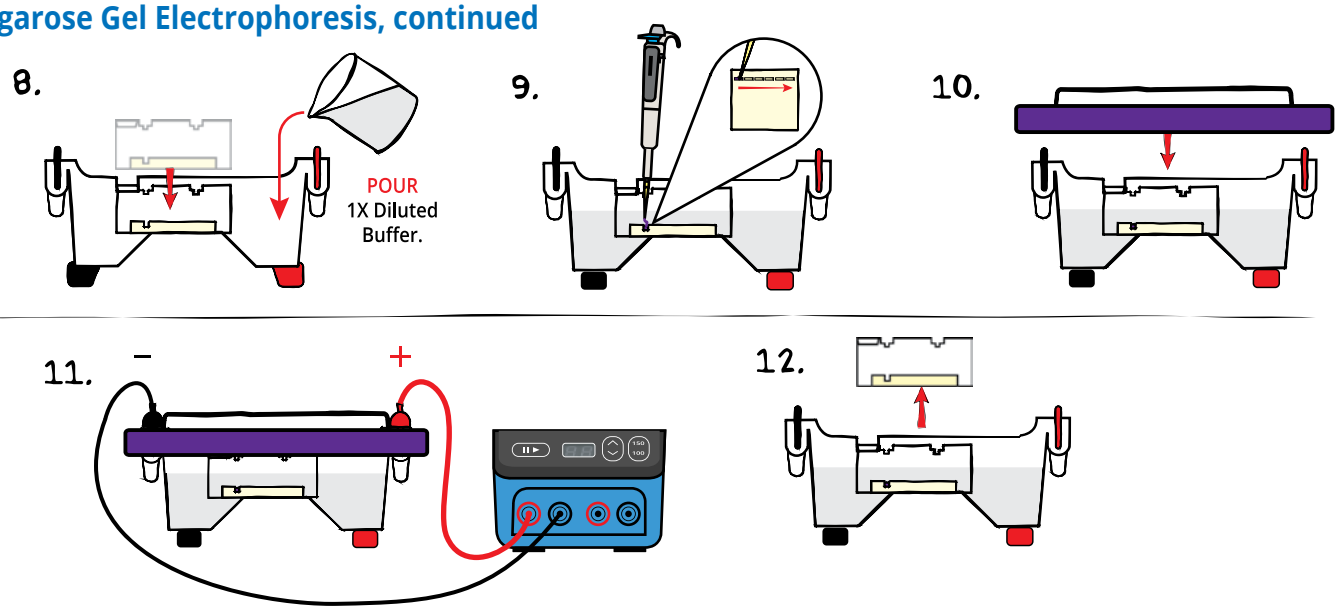
Agarose Gel Electrophoresis, continued



RUNNING THE GEL

8. **PLACE** the gel (still on the tray*) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **LOAD** 35 µL of each sample into the wells in the order indicated by Table 1. ***NOTE: For Option One, each group will load all 6 prepared DNA samples. For Option Two, each group will load the peak DNA samples (Tubes X and Y).***
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

PROCEED to Staining Agarose Gels Using FlashBlue™.

REMINDER:
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

TABLE 1: GEL LOADING

Option One		Option Two	
Lane 1	Tube 1	Lane 1	Tube X
2	Tube 2	2	Tube Y
3	Tube 3		
4	Tube 4		
5	Tube 5		

Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

Table C — **Time and Voltage Guidelines (0.8% Agarose Gel)**

	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

*Gels that have previously been removed from their trays should be "anchored" back to the tray with a few drops of molten agarose before placing into the electrophoresis chamber. This will prevent the gels from sliding around in the trays and the chambers.

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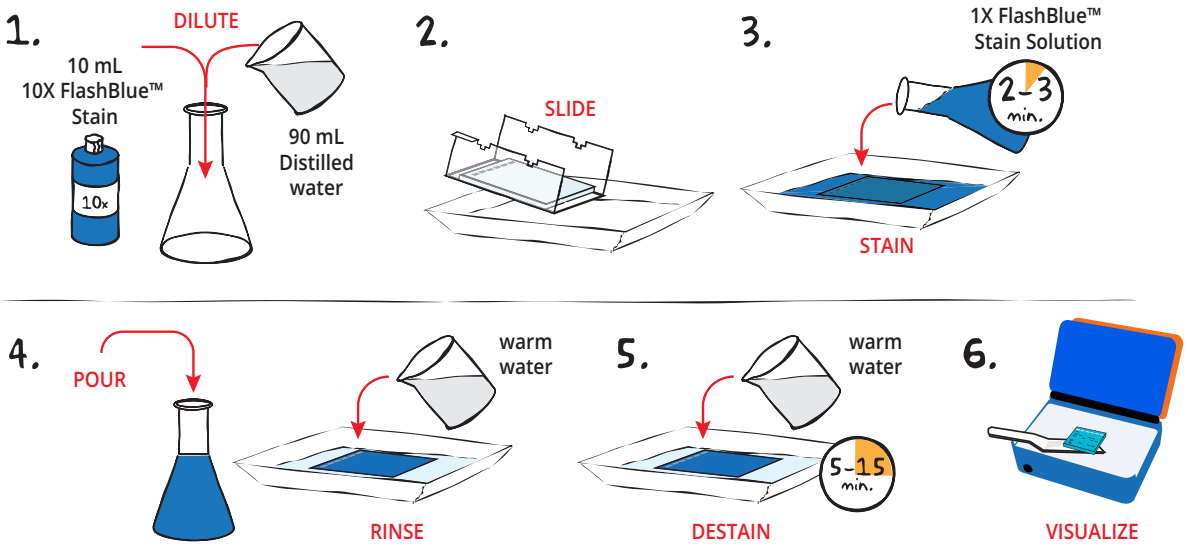
Staining Agarose Gels Using FlashBlue™



1. **DILUTE** 10 mL of 10X concentrated FlashBlue™ with 90 mL of distilled water in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1X FlashBlue™ stain solution. **STAIN** the gel for 2-3 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 3 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **POUR** the 1X FlashBlue™ back into the flask (the stain can be reused). **COVER** the gel with warm water (40-45 °C). Gently **RINSE** the gel for 20-30 seconds. **POUR** off the water.
5. **COVER** the gel with clean, warm water (40-45 °C). **DESTAIN** for 5-15 minutes with gentle shaking (longer periods will yield better results). DNA bands will start to appear after 5 minutes of destaining. Changing the water frequently will accelerate destaining.
6. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Wear gloves and safety goggles

ALTERNATIVE FLASHBLUE™ STAINING PROTOCOL:

1. **DILUTE** 1 mL of 10X FlashBlue™ stain with 149 mL distilled water.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
4. Carefully **REMOVE** the gel from the staining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

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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 10^8?

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.

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Instructor's Guide

HOW THIS EXPERIMENT IS ORGANIZED

This experiment 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 One should be performed if students will conduct electrophoresis of all samples prepared from column fractions collected from the chromatography.

2. Procedure Option Two 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).

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.

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Pre-Lab Preparations

CHROMATOGRAPHY

FOR CHROMATOGRAPHY,
Each lab group will need:

1	Column attached to a ring stand	
12	Microtest tubes	
2	Transfer pipets	
1	Empty beaker	
1	5 or 10 mL pipet	
1	Pipette pump or bulb	
1	Beaker with matrix	5 mL
1	Beaker of elution buffer	50 mL
1	Microtest tube of sample	0.12 mL
1	Microtest tube of water	0.5 mL

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.

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.

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.

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Pre-Lab Preparations

AGAROSE GEL ELECTROPHORESIS

FOR ELECTROPHORESIS, Each lab group will need:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- Chromatography Samples

This experiment requires 0.8% agarose gels. Enough reagents are provided to cast either eight 7 x 7 cm gels, eight 10 x 7 cm gels, or four 14 x 7 cm gels. You can choose whether to prepare the gels in advance or have students prepare their own. Allow approximately 30 minutes for this procedure.

Individual Gel Preparation:
Each student group can be responsible for casting their own individual gel prior to conducting the experiment. Reference the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

Batch Gel Preparation:
To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk. See Appendix B.

Preparing Gels in Advance:
Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at -20 °C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

STAINING AGAROSE GELS USING FLASHBLUE™

FOR STAINING, Each lab group will need:

- 10 mL 10X concentrated FlashBlue *OR* 100 mL 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

FlashBlue™ stain is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to "equilibrate" in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (**Cat. #552**) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

Photodocumentation of DNA (Optional)

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

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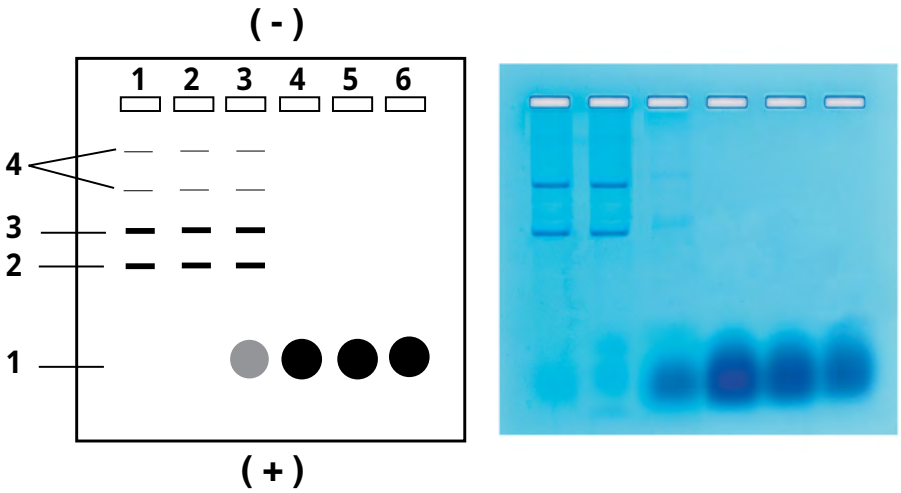
Idealized Schematic of Results



1. RNA
2. Plasmid (single)
3. Plasmid Dimer
4. Higher Catenanes (may not be visible with FlashBlue™)

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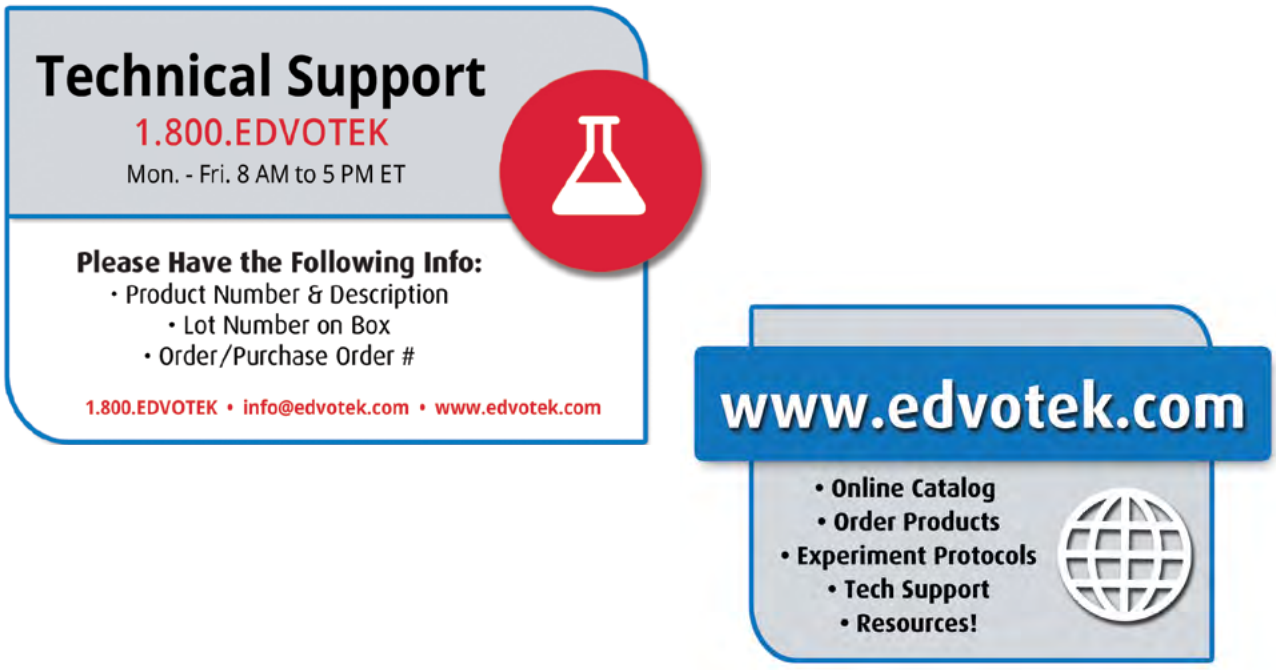
Please refer to the kit insert for the Answers to Study Questions

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Electrophoresis Buffer and Agarose Gels

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



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Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The gel was not stained properly.	Repeat staining.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
	The background of gel is too dark.	Destain the gel for 5-10 minutes in distilled water.
DNA bands were not resolved.	Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).
DNA bands fade when gels are kept at 4 °C.	DNA stained with FlashBlue™ may fade with time.	Re-stain the gel with FlashBlue™.
There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.	The wrong percent gel was used for electrophoretic separation.	Be sure to prepare the correct percent agarose gel. For reference, the Ready-to-Load™ DNA samples should be analyzed using a 0.8% agarose gel.
There's not enough sample in my QuickStrip™.	The QuickStrip™ has dried out.	Add 40 µL water, gently pipet up and down to mix before loading.

Visit www.edvotek.com for additional troubleshooting suggestions.

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Appendix B

Bulk Preparation of Electrophoresis Buffer and Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

Table D

Bulk Preparation of Electrophoresis Buffer			
50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 mL		2,940 mL	3000 mL (3 L)

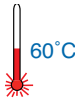
Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

Table E

Batch Prep of 0.8% UltraSpec-Agarose™				
Amt of Agarose	+	Concentrated Buffer (50X)	+ Distilled Water	Total Volume
3.0 g		7.5 mL	367.5 mL	375 mL

1. Use a 500 mL flask to prepare the diluted gel buffer.
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60 °C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 mL for a 7 x 7 cm tray, 45 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 cm tray. ***For this experiment, 7 x 7 cm gels are recommended.***
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks. Do not freeze gels.

NOTE:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

PROCEED to Loading and Running the Gel (page 12).

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