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Edvo-Kit #

Edvo-Kit #118

Cholesterol Diagnostics

Experiment Objective:

In this experiment, students will use agarose gel electrophoresis to explore the genetics of familial hypercholesterolemia and the molecular methods used to identify this disease.

See page 3 for storage instructions.

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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

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Cor Sto	nponents (in QuickStrip™ format) re QuickStrip™ samples in the refrigerator upon receipt.	Check (🗸)
А	DNA Standard Marker	
В	Normal DNA Sample	
С	FH Control	
D	Patient #1 DNA Sample	
Е	Patient #2 DNA Sample	
F	Patient #3 DNA Sample	
RE	AGENTS & SUPPLIES	

Experiment #118 is designed for 8 groups.

Store QuickStrip[™] samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

Store the following at room temperature.

•	UltraSpec-Agarose™	
•	Electrophoresis Buffer (50x)	
•	Practice Gel Loading Solution	
•	FlashBlue™ DNA Stain	

FlashBlue[™] DNA Stain

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 or deionized water

All experiment 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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Cholesterol

Background Information

Cholesterol is a complex lipid essential for the survival of all animal cells. Its primary function is the stabilization of plasmer and organellar membranes. The structure of cholesterol, shown in Figure 1, demonstrates the four-ring backbone found in steroid hormones such as testosterone and estrogens. Cholesterol is also a precursor for vitamin D and for bile salts, which facilitate the digestion of lipids in the intestine.

Cholesterol is synthesized in the liver and is absorbed in the intestine from ingested food. It is circulated in body fluids in spherical bodies known as lipoprotein particles. These lipoproteins are classified according to their

densities determined by gradient centrifugation. Cholesterol processed by the liver is packaged into particles known as very low-density lipoproteins (VLDL), which are processed in the bloodstream to form low-density lipoproteins (LDL). High-density lipoproteins (HDL) take up cholesterol from LDL and peripheral tissues and transport it back to the liver for repackaging or excretion (Figure 2). Because HDL removes cholesterol from the circulation, this is often referred to as "good cholesterol". LDL, in contrast, transport cholesterol from the liver to arteries and is often termed "bad cholesterol".

While cholesterol is essential for life, excess serum cholesterol can have serious

negative consequences. The role of elevated blood cholesterol (especially LDL) in cardiovascular disease is well established. LDL can accumulate on arterial walls; LDL is then oxidized by molecules known as *free radicals* that are released from arterial wall membranes. This oxidation process results in the accumulation of inflammatory cells, resulting in the formation of a fatty substance known as plaque. Amassing of plaque can eventually lead to occlusions that restrict blood flow to the heart or brain, resulting in heart attack or stroke.

As mentioned, most circulating cholesterol is found in LDL particles. Animal cells take up LDL from the circulation by a specific receptor. Individuals with a condition known as familial hypercholesterolemia (FH) possess mutations in the gene for the LDL receptor and thus are unable to efficiently remove LDL from the circulation. The result of this deficient uptake is that LDL remains in the circulation and accumulates on arterial walls. Patients who are heterozygous for this mutation still have one functional gene and therefore possess 50% the normal level of receptors of unaffected individuals. Patients who are homozygous for the mutation, however, completely lack the LDL receptor and therefore possess extremely elevated levels of serum cholesterol, often greater than 600 mg per 100 ml of serum (150-200 mg per 100 ml of serum is considered normal). These patients, if untreated, usually die in childhood of coronary artery disease.

FH results from any of hundreds of different mutations in the FH gene. In some ethnic groups, however, the disease may be passed on as a single mutation. The mutation may be detected by a combination of genetic-based diagnostics, such as Polymerase Chain Reaction (PCR) along with Restriction Fragment Length Polymorphism (RFLP) analysis.



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Figure 3: DNA Amplification by the Polymerase Chain Reaction

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In PCR, a specific region of DNA, usually within or near a disease-causing gene, is first amplified. PCR (Figure 3) uses an enzyme known as *Taq* DNA polymerase. This enzyme, purified from a bacterium that is found in hot springs, is stable at high temperatures. In the first step of PCR (denaturation), DNA complimentary strands are separated at 94 °C, while the *Taq* DNA polymerase remains stable. In the second step (annealing), the sample is cooled to a temperature between 42 °C and 65 °C. This "cooling" allows hybridization of a set of two small (15-30) synthetic oligonucleotides, known as "primers", to the target region to be amplified. In this experiment, the target is the FH gene. In the third step (extension), the temperature is raised to 72 °C and the *Taq* DNA polymerase then adds nucleotides to the primers to complete each new complimentary strand of the target. These three steps constitute one PCR "cycle". This process is typically repeated from 25-40 cycles, amplifying the target exponentially (Figure 3). PCR is performed in a thermal cycler, which is programmed to heat or cool the PCR reaction at the designated temperature for each step.

Typically, following PCR of the target region within a gene, the amplified DNA is further examined using RFLP analysis. An RFLP is defined as a variation in the number of restriction sites in a specific DNA region in diseased vs. healthy individuals. The amplified DNA is digested with a specific restriction enzyme. If the patient possesses a mutation in the LDL receptor gene, the digestion pattern will differ from the pattern obtained from unaffected individuals.

In this experiment, simulated DNA samples from three patients will be analyzed for the FH mutation. Each sample has previously been amplified and digested with a restriction enzyme and is ready to be separated by electrophoresis on an agarose gel. From the stained gel, one can determine whether the patients are normal, heterozygous, or homozygous for the FH mutation. DNA from patients who are heterozygous for the mutation will reveal one normal and one mutated allele, as shown in Figure 4. Homozygous individuals for the mutation will possess two mutated alleles.

DNA Standard Marker Normal DNA FH Heterozygote FH Homozygote	
=	
Figure 4:	-





Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will use agarose gel electrophoresis to explore the genetics of familial hypercholesterolemia and the molecular methods used to identify this disease.

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.

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.





Experiment Overview



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Module I: 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).
- 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.
- 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.

Table A	In	dividual 0.89	% UltraSpe	c-Agaros	e™ Gels
Size c Castin	of Gel Ng tray	Concentrated Buffer (50x)	Distilled + Water +	Ant of Agarose	= TOTAL Volume
7 x 7	7 cm	0.6 mL	29.4 mL	0.24 g	30 mL
10 x 7	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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Module I: Agarose Gel Electrophoresis



RUNNING THE GEL

- 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. **PUNCTURE** the foil overlay of the QuickStrip[™] with a pipet tip. **LOAD** the entire sample (35 μL) into the well in the order indicated by Table 1, at right.
- 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.

1x Electrophoresis Buffer (Chamber Buffer)

TABLE 1: GEL LOADING				
Lane 1 Tube A		DNA Standard Marker		
2	2 Tube B Normal DNA Sample			
3 Tube C		FH Control		
4	Tube D	Patient #1 DNA Sample		
5 Tube E Patient #2 DNA Sample		Patient #2 DNA Sample		
6	6 Tube F Patient #3 DNA Sample			

Table C	Time and Voltage Guidelines (0.8% Agarose Gel)			
	Electrophe EDGE™	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		

PROCEED to Module II: Staining Agarose Gels Using FlashBlue[™].

total Volume

Required

150 mL

400 mL

1000 mL

*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 in	nto the
electrophoresis chamber. This will prevent the gels from sliding around in the trays and the chambers.	

Distilled

Water

147 mL

392 mL

980 mL

Dilution

50x Conc.

Buffer

3 mL

8 mL

20 mL



Table

В

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Model #

EDGE™

M12 M36

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REMINDER: Before loading the

samples, make sure the gel is properly oriented in the apparatus chamber.

Module II: 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.

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.



Wear gloves

and safety goggles

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Module III: Data Analysis Using a Standard Curve

Agarose gel electrophoresis separates biomolecules into discrete bands, each comprising molecules of the same size. How can these results be used to determine the lengths of different fragments? Remember, as the length of a biomolecule increases, the distance to which the molecule can migrate decreases because large molecules cannot pass through the channels in the gel with ease. Therefore, the migration rate is inversely proportional to the length of the molecules—more specifically, to the log10 of molecule's length. To illustrate this, we ran a sample that contains bands of known lengths called a "standard". We will measure the distance that each of these bands traveled to create a graph, known as a "standard curve", which can then be used to extrapolate the size of unknown molecule(s).

1. Measure and Record Migration Distances

Measure the distance traveled by each Standard DNA Fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard.

Measure and record the migration distances of each of the fragments in the unknown samples in the same way you measured the standard bands.

2. Generate a Standard Curve

Because migration rate is inversely proportional to the log10 of band length, plotting the data as a semi-log plot will produce a straight line and allow us to analyze an exponential range of fragment sizes. You will notice that the vertical axis of the semi-log plot appears atypical at first; the distance between numbers shrinks as the axis progresses from 1 to 9. This is because the axis represents a logarithmic scale. The first cycle on the y-axis corresponds to lengths from 100-1,000 base pairs, the second cycle measures 1,000-10,000 base pairs, and so on. To create a standard curve on the semi-log paper, plot the distance each Standard DNA fragment migrated on the x-axis (in mm) versus its size on the y-axis (in base pairs). Be sure to label the axes!



Figure 5:

Measure distance migrated from the lower edge of the well to the lower edge of each band.





Module III: Data Analysis Using a Standard Curve

After all the points have been plotted, use a ruler or a straight edge to draw the best straight line possible through the points. The line should have approximately equal numbers of points scattered on each side of the line. It is okay if the line runs through some points (see Figure 6 for an example).

3. Determine the length of each unknown fragment.

- a. Locate the migration distance of the unknown fragment on the x-axis of your semi-log graph. Draw a vertical line extending from that point until it intersects the line of your standard curve.
- From the point of intersection, draw a second line, this time horizontally, toward the y-axis. The value at which this line intersects the y-axis represents the approximate size of the fragment in base pairs (refer to Figure 6 for an example). Make note of this in your lab notebook.
- c. Repeat for each fragment in your unknown sample.



Quick Reference:

DNA Standard fragment sizes - length is expressed in base pairs.

6751, 3652, 2827, 1568, 1118, 825, 630





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Study Questions

- 1. What is familial hypercholesterolemia (FH)? If both parents of a child are heterozygous for FH, what is the probability that the child will be mildly afflicted with the disease? What are the chances that the child will be severely afflicted?
- 2. What is RFLP analysis? How can RFLPs be used to screen for a genetic disease?
- 3. Describe the differences between LDL and HDL. Does it make any difference (to one's health) whether cholesterol is found in LDL or HDL? Why?
- 4. Why is it important to avoid having high blood cholesterol levels? What are some possible causes of high cholesterol levels?
- 5. What is a statin?



Instructor's Guide

ADVANCE PREPARATION:

PREPARATION FOR:	WHAT TO DO: WHEN?		TIME REQUIRED:	
	Prepare QuickStrips™.			
Module I: Agarose Gel	Prepare diluted electrophoresis buffer.	Up to one day before performing	45 min.	
Electrophoresis	Prepare molten agarose and pour gels.	the experiment.		
Module II: Staining Agarose Gels Using FlashBlue™	Prepare staining components.	The class period or overnight after the class period.	10 min.	





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Pre-Lab Preparations: Module I

AGAROSE GEL ELECTROPHORESIS

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.

Quick Reference for EDVO-Kit #103					
Size of gel Groups Placement of Wells ro casting tray per gel comb per g			Wells required per group		
7 x 7 cm 1 group		1st set of notches	6		
10 x 7 cm 1 group		1st set of notches	6		
14 x 7 cm 2 groups		1st and 3rd sets of notches	6		

FOR MODULE I Each group will need:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- QuickStrip[™] Samples

NOTE:
This kit is compatible with
<u>SYBR[®] Safe Stain</u>
(Cat #608, not included).
Instructions for preparing gels
and visualizing
results can be found
in Appendix C.

Individual Gel Preparation:

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module I in 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.

SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

QuickStrip[™] tubes consist of a microtiter block covered with a protective foil overlay. Each well contains pre-aliquoted sample.

Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the foil overlay while separating the samples.

Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube. Puncture the foil overlay of the QuickStrip[™] with a pipet tip to aspirate the sample. *Do not remove the foil as samples can spill.*



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Pre-Lab Preparations: Module II

STAINING AGAROSE GELS USING FLASHBLUE™

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.

FOR MODULE II Each group will need:

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

NOTE:

Accurate pipetting is critical for maximizing successful experiment results. EDVOTEK Series 100 experiments are designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students are unfamiliar with using micropipettes, we recommended performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this experiment.



Experiment Results and Analysis



Includes EDVOTEK's All-NEW DNA Standard Marker • Better separation • Easier band measurements • No unused bands NEW DNA Standard ladder sizes: 6751, 3652, 2827, 1568, 1118, 825, 630

In the idealized schematic, the relative positions of DNA fragments are shown but are not depicted to scale.

Lane	Tube	Sample	Result	Molecular Weights (in bp)
1	A	DNA Standard Markers		6751, 3652, 2827, 1568 1118, 825, 630
2	В	Normal Control Sample	FH Normal	4282
3	С	FH Control	Homozygous for FH mutation	3000, 1282
4	D	Patient #1	FH Normal	4282
5	E	Patient #2	Heterozygous for FH mutation	4282, 3000, 1282
6	F	Patient #3	Homozygous for FH mutation	3000, 1282



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Questions and Answers to Study Questions

1. What is familial hypercholesterolemia (FH)? If both parents of a child are heterozygous for FH, what is the probability that the child will be mildly afflicted with the disease? What are the chances that the child will be severely afflicted?

Familial hypercholesterolemia (FH) is a dominant genetic disease in which one or more mutations exist in the gene encoding the low-density lipoprotein (LDL) receptor. As the mutation is dominant, a child born of parents who are both affected would have a 75% chance of being at least mildly afflicted (heterozygous) and a 25% chance of being severely afflicted (homozygous).

2. What is RFLP analysis? How can RFLPs be used to screen for a genetic disease?

RFLP stands for Restriction Fragment Length Polymorphism. This technique relies on a difference in the number of restriction sites that are correlated with a certain disease. To perform RFLP analysis, total genomic DNA is digested with a specific restriction enzyme and electrophoresed on a gel. A restriction enzyme mutation at the cutting site will change the patient's DNA digest pattern. The DNA from the gel is transferred to a membrane and hybridized to a probe to a specific region of a chromosome.

3. Describe the differences between LDL and HDL. Does it make any difference (to one's health) whether cholesterol is found in LDL or HDL? Why?

LDL stands for low-density lipoprotein, spherical particles that carry cholesterol from the liver to the peripheral tissues. Because LDL is the form in which most circulating cholesterol is found, it is often referred to as "bad cholesterol". HDL stands for high-density lipoprotein, particles that ferry cholesterol from peripheral tissues back to the liver for repackaging or for excretion. As HDL particles tend to remove cholesterol from the circulation, cholesterol found in this form is often referred to as "good cholesterol".

4. Why is it important to avoid having high blood cholesterol levels? What are some possible causes of high cholesterol levels?

Excess cholesterol can accumulate inside and within arterial walls in occlusions known as plaques. Sufficient plaque formation will result in complete artery blockage, resulting in heart attack or stroke. The American Heart Association recommends a total blood cholesterol level of less than 200 mg/dL and an LDL level less than 130 mg/dL. A deciliter (dL) is a tenth (100 ml) of a liter. In addition to diet, diseases such as the FH disorder, in which excess cholesterol is synthesized, may cause high serum cholesterol levels.

5. What is a statin?

Statins are a recently discovered class of drugs that inhibit cholesterol synthesis in the liver and thus lower blood cholesterol levels. Besides heart disease, statins may also lower the risk of kidney and Alzheimer's disease.



Appendices

- A EDVOTEK® Troubleshooting Guide
- B Bulk Preparation of Electrophoresis Buffer and Agarose Gels
- C Using SYBR® Safe Stain (OPTIONAL)

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





Appendix A

EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:		
	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.		
Bands are not visible on the gel.	The gel was not stained properly.	Repeat staining protocol.		
<u>-</u>	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.		
After staining the gel.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.		
the DNA bands are faint.	The background of gel is too dark after staining with FlashBlue™.	Destain the gel for 5-10 minutes in distilled water.		
DNA bands were not resolved.	Tracking dye should migrate at least 3 cm from the wells to ensure adequate separation.	Be sure to run the gel at least 3 cm before staining and visualizing the DNA (approximately 15-20 minutes at 150 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 <u>www.edvotek.com</u> 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.

Batch Agarose Gels (0.8%)

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

- 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.

PROCEED to Loading and Running the Gel (page 10).

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





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.



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Appendix C

Using SYBR[®] Safe DNA Stain (OPTIONAL)

If desired, the DNA samples in this experiment can be visualized using <u>SYBR® Safe DNA stain (Cat #608)</u>. We recommend adding diluted SYBR® Safe stain to the liquid agarose gels while casting for easy, reproducible results. A blue light or UV transilluminator is needed for visualizing SYBR® gels. The TruBlu™ 2 (<u>Cat. #557</u>) is highly recommended.

PREPARING SYBR® SAFE STAIN

Instructors:

- 1. Prepare 1x Electrophoresis Buffer by combining 10 μL of 50X Concentrated Buffer with 490 μL of distilled water.
- 2. Add 20 µL of the SYBR[®] Safe to the tube of 1X buffer from Step 1 and mix by tapping the tube several times. The diluted SYBR[®] Safe Stain is now ready to be used during agarose gel preparation.

AGAROSE GEL PREPARATION

This experiment requires one 0.8% agarose gel for each student group. Instructors can choose whether to prepare the gels in advance (METHOD A) or have the students prepare their own (METHOD B). Allow approximately 30-40 minutes for this procedure.

Instructor Preparation (METHOD A):

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

- 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. Add the entire tube of *diluted* **SYBR® Safe** stain to the cooled agarose and mix well.

- 7. 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.*
- 8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Solidified gels can be stored in the refrigerator for up to 2 weeks. Place 1-2 mL of electrophoresis buffer in a sealable bag with the gels to prevent them from drying out. Excessive buffer will cause SYBR[®] Safe to diffuse out of the gels. Do not freeze gels.

PROCEED to Loading and Running the Gel (Steps 8-12 on page 10), followed by the VISUALIZATION procedures on page 26. **NO ADDITIONAL STAINING IS NECESSARY.**



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Ta	ble	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	

60°C

Appendix C Using SYBR[®] Safe DNA Stain (OPTIONAL)

AGAROSE GEL PREPARATION, CONTINUED

Student Preparation (METHOD B):

For student preparation of agarose gels, see Table A.2.

- 1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A.2).
- 2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).

_												
Table A.2		Inc	Individual 0.8% UltraSpec-Agarose™ with SYBR® Stain									
	Size of Gel Casting tray 7 x 7 cm 10 x 7 cm* 14 x 7 cm		Concentrated Buffer (50x)	+ Distilled + Water +	Amt of Agarose	= TOTAL Volume	Diluted SYBR® (Step 6)					
			0.6 mL	29.4 mL	0.24 g	30 mL	30 µL					
			0.9 mL	44.1 mL	0.36 g	45 mL	45 µL					
			1.2 mL	58.8 mL	0.48 g	60 mL	60 µL					

^{3.} **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. * *Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*

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. Before casting the gel, **ADD** <u>diluted</u> SYBR[®] Safe to the cooled agarose and swirl to mix (see Table A.2).
- 7. **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.
- 8. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

PROCEED to Loading and Running the Gel (Steps 8-12 on page 10), followed by the VISUALIZATION procedures on page 26. **NO ADDITIONAL STAINING IS NECESSARY.**



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60°C

Appendix C Using SYBR[®] Safe DNA Stain (OPTIONAL)

VISUALIZING THE SYBR® GEL

A blue light or UV transilluminator is needed for visualizing SYBR® gels. The TruBlu™ 2 (Cat. #557) is highly recommended.



- 1. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator.
- 2. Turn the unit **ON**. DNA should appear as bright green bands on a dark background. **PHOTOGRAPH** results.
- 3. Turn the unit **OFF. REMOVE** and **DISPOSE** of the gel. **CLEAN** the transilluminator surfaces with distilled water.



Be sure to wear UV goggles if using a UV transilluminator.

