EDVOTEK® QUICK GUIDE

Biotechnology Basics: Innovations In Your Classroom





Biotechnology represents the use of cellular, molecular, and biochemical technologies to improve society and the environment. Basic biotechnology techniques have been used for centuries for the production and preservation of food, selective breeding of livestock, and to improve human health. Modern laboratories that discover many of these biotechnological innovations use simple but powerful techniques to visualize and manipulate DNA.

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Polymerase Chain Reaction (PCR)

WHAT IS PCR?

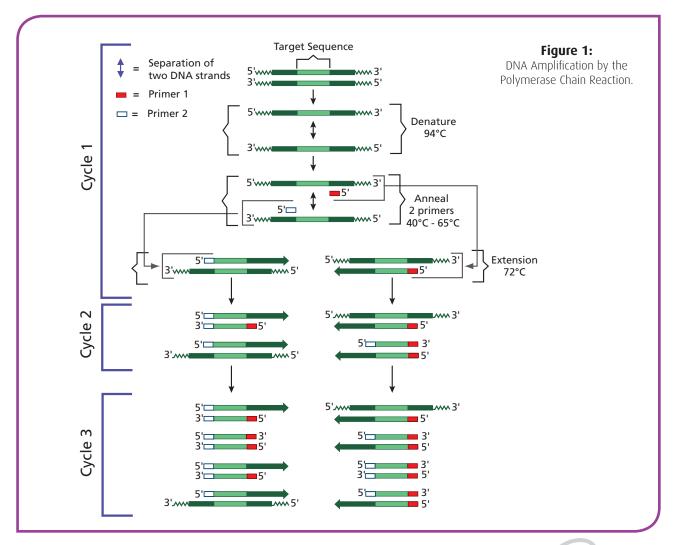
PCR is a technique that allows researchers to quickly create many copies of a specific region of DNA *in vitro*.

WHAT DO I NEED TO PERFORM PCR?

- Template the purified, double-stranded piece of DNA we want to copy
- Primers short synthetic DNA molecules that target a specific DNA sequence for amplification
- Taq DNA Polymerase thermostable enzyme used to copy DNA
- Free nucleotides the building blocks of DNA
- Thermal Cycler (a.k.a. PCR machine) a specialized machine that rapidly heats and cools the samples.

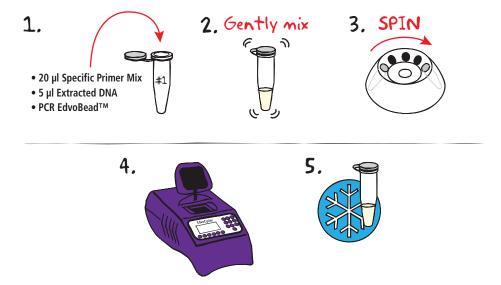
HOW DOES PCR WORK?

To perform PCR, the template is mixed with primers, *Taq* polymerase and nucleotides. The mixture is heated to 94° C to denature the DNA duplex (i.e. unzip it into single strands). Next, the sample is cooled to 45°-60° C, allowing the primers to base pair with the target DNA sequence (called "annealing"). Lastly, the temperature is raised to 72° C, the optimal temperature at which *Taq* polymerase will extend the primer to synthesize a new strand of DNA. Each "PCR cycle" (denaturation, annealing, extension) doubles the amount of the target sequence in less than five minutes. In order to produce enough DNA for analysis, twenty to forty cycles may be required.

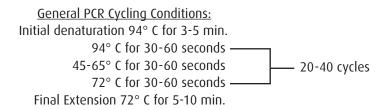


EDVOTEK®

Polymerase Chain Reaction (PCR)



- 1. **ADD** 20 μl specific primer mix, 5 μl extracted DNA and the PCR EdvoBead™ to a labeled 0.2 ml tube.
- 2. **MIX** the PCR sample. Make sure the PCR EdvoBead™ is completely dissolved.
- 3. **CENTRIFUGE** the sample for a few seconds to collect the sample at the bottom of the tube.
- 4. **AMPLIFY** DNA using PCR.



6. **PLACE** tubes on ice. Analyze samples using agarose gel electrophoresis.



Agarose Gel Electrophoresis

WHAT IS ELECTROPHORESIS?

Electrophoresis is a technique that allows us to separate DNA, RNA or proteins according to their size.

WHAT DO I NEED TO SEPARATE A MIXTURE OF DNA MOLECULES?

In addition to your DNA sample, you will need:

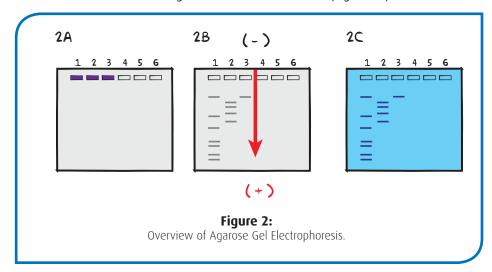
- Gel Loading Solution includes glycerol to help DNA samples enter into the wells and a visible dye to monitor migration through the gel.
- **Agarose** a polysaccharide used as the separation matrix.
- Electrophoresis Buffer contains ions necessary to conduct an electrical current, maintains pH of experiment.
- Horizontal electrophoresis apparatus holds the buffer and the gel, has positive and negative electrodes.
- Power supply generates the current necessary to move DNA through gel.
- **Micropipet** used to transfer samples into wells.
- A special stain that allows us to visualize DNA.



HOW DOES ELECTROPHORESIS SEPARATE DNA FRAGMENTS?

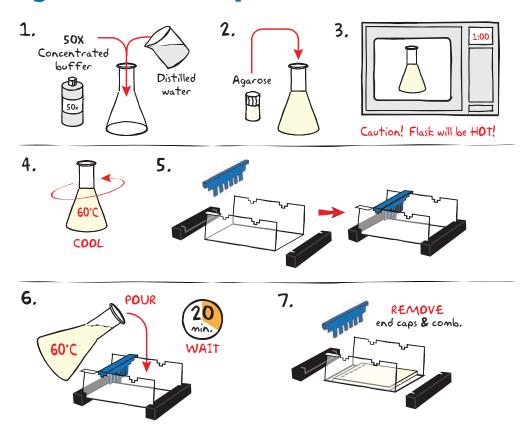
The mixture of DNA molecules is added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel (Figure 2A). Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode (Figure 2B).

At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA (Figure 2C).





Agarose Gel Electrophoresis



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

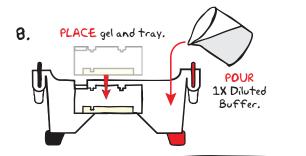


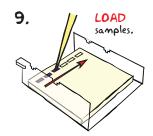
- 4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
- 5. While agarose is cooling, **SEAL** the ends of the gelcasting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
- 6. **POUR** the cooled agarose solution into the prepared 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.

Table A	Individual 0.8% UltraSpec-Agarose™ Gel				
	of Gel ig tray	Concentrated Buffer (50x)	Distilled + Water +	Ant of Agarose =	†OTAL Volume
7×1	7 cm	0.6 ml	29.4 ml	0.2 3 g	30 ml
7×1	0 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7×1	4 cm	1.2 ml	58.8 ml	0.46 g	60 ml

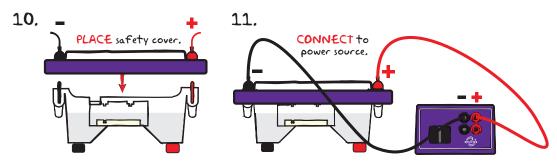


Agarose Gel Electrophoresis









- 8. **PLACE** gel (on the tray) into electrophoresis chamber. Completely **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes).
- 9. **LOAD** entire sample volumes into wells in consecutive order.
- 10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the 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).
- 12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING & VISUALIZATION**.

Reminder:

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

	table B				
		EDV O TEK Model #	Total Volume Required	Dilut 50x Conc. Buffer	
	M6+ & M12 (new) M12 (classic)		300 ml	6 ml	294 ml
			400 ml	8 ml	392 ml
		M36	1000 ml	20 ml	980 ml

	Table	Time and Voltage Guidelines (0.8% Agarose Gel)		
ī	Electrophoresis Model			
		M6+	M12 (new)	M12 (classic) & M36
	Volts	Min./Max.	Min./Max.	Min./Max.
	150	15/20 min.	20/30 min.	25 / 35 min.
	125	20/30 min.	30/35 min.	35 / 45 min.
	75	35 / 45 min.	55/70 min.	60/90 min.



Visualizing DNA

Agarose gel electrophoresis is used to separate DNA fragments in complex mixtures according to their size. However, because DNA is clear and colorless, these bands cannot be seen with the naked eye. Edvotek® offers several different methods for visualizing the DNA separated by electrophoresis.

FLUORESCENT DNA STAINS:

Research laboratories commonly use fluorescent DNA stains because they are extremely sensitive, making it easy to quantify small amounts of DNA. In order to visualize the DNA fragments, an ultraviolet (UV) light source (such as a transilluminator) is used to excite the fluorescent molecules. We offer two fluorescent DNA stains: InstaStain® Ethidium Bromide and SYBR® Safe DNA Stain.

VISIBLE DYE-BASED DNA STAINS:

Although they are less sensitive than fluorescent stains, dye-based DNA stains are an excellent alternative for the teaching classroom, as they are non-toxic and require no special equipment for visualization. The molecules of the DNA stain possess a positive charge, which allows them to bind to the negatively charged backbone of DNA. The DNA fragments are easily visualized because the bound dye molecules stain them with an intense blue color. We offer two visible dye-based DNA Stains: InstaStain® Blue and Flash Blue Stain.

InstaStain® Ethidium Bromide SYBR® Safe DNA Stain

InstaStain® Blue and FlashBlue™



WHICH DNA STAIN SHOULD I USE?

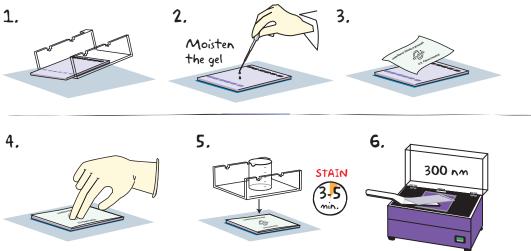
Stain	Advantages	Disadvantages
InstaStain® EtBr	Very sensitive Very fast	Requires UV transilluminator Potentially mutagenic
SYBR® Safe	Very sensitive Non-mutagenic	Requires UV transilluminator More expensive
InstaStain® Blue	Easy to use Generates minimal waste	Less sensitive More time
FlashBlue™	Simple and fast Reusable, inexpensive	Less sensitive Disposal of liquid



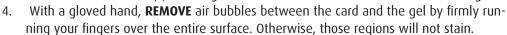
Visualizing DNA with InstaStain® Ethidium Bromide

INSTASTAIN® ETHIDIUM BROMIDE

The most commonly used fluorescent DNA stain is Ethidium Bromide (EtBr). Individual EtBr molecules can squeeze between neighboring base pairs in a DNA double helix in a process known as "intercalation". When excited with UV light, any EtBr intercalated into the DNA fluoresces and produces a bright orange light. However, because EtBr is a potential mutagen, it must be handled with care. InstaStain® Ethidium Bromide provides the sensitivity of EtBr while minimizing potential contact with hazardous materials by delivering a small amount of stain to the agarose gel via a special paper backing.



- 1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface. **DO** NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.
- **MOISTEN** the gel with a few drops of electrophoresis buffer.
- Wearing gloves, **REMOVE** and **DISCARD** the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). **PLACE** the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. You will need 2 cards to stain a 7 x 14 cm gel.



- **PLACE** the casting tray on top of the qel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. **STAIN** the gel for at least 3-5 minutes. For best results, the gel should be stained for 15 minutes.
- **REMOVE** the InstaStain® Ethidium Bromide card(s). **VISUALIZE** the gel using a mid-range ultraviolet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!



Wear gloves and

UV safety goggles

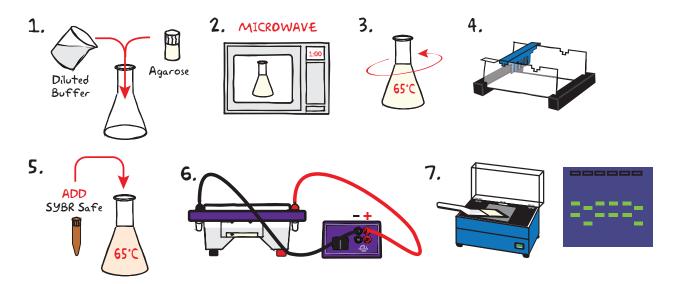
Visualizing DNA with SYBR® Safe Stain

METHOD I: IN-GEL SYBR® SAFE DNA STAINING PROTOCOL (PREFERRED METHOD)

This fast, easy staining protocol incorporates SYBR® Safe into the molten agarose before the gel is poured into the casting tray. This means that the DNA is staining while the electrophoresis experiment is running! Results can be visualized immediately post electrophoresis.

SYBR® Safe is provided as a 10,000X concentrate. Be sure to calculate the amount used for staining before casting the gel. For example, 5 µl of SYBR® Safe is added to 50 ml of molten agarose for DNA visualization.

Agarose gels may be prepared in advance and stored for later use. Place gels in a plastic container and cover with 1X Electrophoresis Buffer containing SYBR® Safe at a 1:10,000 dilution. Store in the dark at 4° C for up to a week.



- 1. MIX diluted electrophoresis buffer and agarose powder as specified in your experimental protocol. **DISSOLVE** agarose powder by boiling the solution.
- 2. **MICROWAVE** the solution on high for one minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in thirtysecond bursts until the agarose is completely melted (the solution should be clear like water).



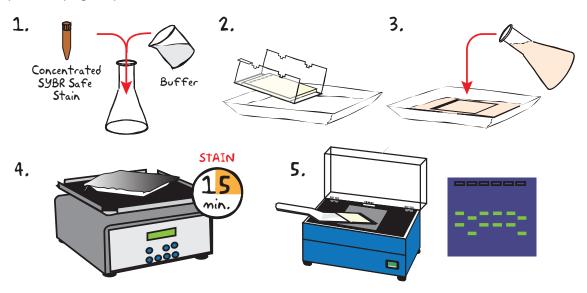
- 3. **COOL** the molten agarose to 65° C with careful swirling to promote even dissipation of
- 4. **PREPARE** gel-casting tray while the gel is cooling.
- 5. Before casting the gel, ADD SYBR® Safe concentrate to the molten agarose and swirl to mix well. The agarose solution may appear pale orange in color.
- 6. **PERFORM** electrophoresis as specified in your experimental protocol. To avoid dye-front migration issues on long gels (>10 cm), we recommend adding SYBR® Safe to the Electrophoresis Buffer at a 1:10,000 dilution. Gels under 7 cm in length should not be affected.
- 7. After electrophoresis is complete, **REMOVE** gel and casting tray from the electrophoresis chamber. Carefully **SLIDE** gel off of the casting tray onto the viewing surface of the transilluminator and turn the unit on. DNA should appear as bright green bands on a dark background.



Visualizing DNA with SYBR® Safe Stain

METHOD II: POST-ELECTROPHORESIS SYBR® SAFE DNA STAINING PROTOCOL

Run agarose gel(s) as usual according to your standard protocol. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads, and remove the cover.



- 1. **DILUTE** SYBR® Safe 1: 10,000 by adding 7.5 μl of the concentrated stain to 75 ml of 1x electrophoresis buffer in a flask. **MIX** well.
- 2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
- 3. **POUR** the 1x SYBR® Safe stain solution over the gel. **COVER** the gel completely.
- 4. **COVER** the tray with foil to protect the gel from light. **STAIN** the gel for 10-15 minutes. For best results, use an orbital shaker to gently agitate the gel while staining.
- 5. **REMOVE** the gel from the staining solution. **SLIDE** gel off of the casting tray onto the viewing surface of the transilluminator and turn the unit on. DNA should appear as bright green bands on a dark background.

DISPOSAL OF SYBR® SAFE

SYBR® Safe DNA Stain is not classified as hazardous waste, thus can be safely disposed of down the drain or in the regular trash, providing convenience and reducing cost in waste disposal.

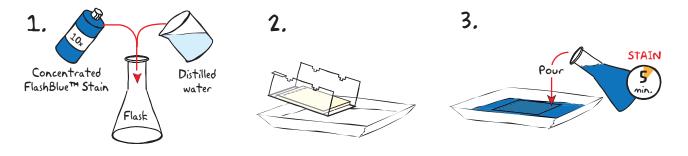


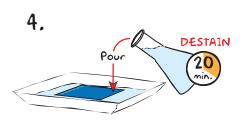


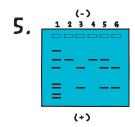
Wear gloves and

UV safety goggles

Visualizing DNA *with FlashBlue™ Liquid Stain*







- 1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
- 2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
- 3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING** THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.



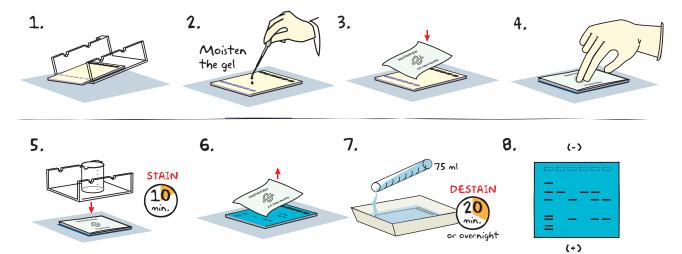
- 4. TRANSFER the gel to a second small tray. COVER the gel with water. DESTAIN for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
- 5. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

ALTERNATIVE PROTOCOL:

- 1. **DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH₂O.
- 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.



Visualizing DNA with InstaStain® Blue



- 1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface.
- 2. **MOISTEN** the gel with a few drops of electrophoresis buffer.
- 3. Wearing gloves, **PLACE** the blue side of the InstaStain® Blue card on the gel.
- 4. With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
- 5. **PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Blue card is in direct contact with the gel surface. **STAIN** the gel for 10 minutes.
- 6. **REMOVE** the InstaStain® Blue card. If the color of the gel appears very light, reapply the InstaStain® Blue card to the gel for an additional five minutes.
- 7. **TRANSFER** the gel to a small, clean gel-staining tray. **COVER** the gel with about 75 mL of distilled water and **DESTAIN** for at least 20 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. To accelerate destaining, warm the distilled water to 37°C and change it frequently.
- 8. 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 PROTOCOL:

- 1. Carefully **SLIDE** the agarose gel from its casting tray into a small, clean tray containing about 75 ml of distilled/deionized water or used electrophoresis buffer. The gel should be completely submerged.
- 2. Gently **FLOAT** the InstaStain® Blue card(s) on top of the liquid with the stain (blue side) facing toward the gel. Each InstaStain® Blue card will stain 49 cm² of gel (7 x 7 cm).
- 3. **COVER** the tray with plastic wrap to prevent evaporation. **SOAK** the gel in the staining liquid for at least 3 hours. The gel can remain in the liquid overnight if necessary.
- 4. Carefully **REMOVE** the gel from the staining tray. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.



NOTE: DO NOT STAIN GELS IN THE ELECTROPHO-RESIS APPARATUS.



PCR - Equipment

Cat. #541

EdvoCycler™

Features a 0.2 ml tube block which has room for up to 25 student samples and comes preprogrammed with all Edvotek PCR kit protocols.

www.edvotek.com/541



ELECTROPHORESIS - Equipment



Cat. #515
M36 HexaGel™ Apparatus
For 6 Lab Groups
www.edvotek.com/515



Cat. #502/504 M12 Complete™ For 2 Lab Groups www.edvotek.com/504



Cat. #500
M6 Plus™ Apparatus
For 1 Lab Group
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Cat. #509 DuoSource™ 150 75/150 V for 1 or 2 units www.edvotek.com/509



Cat. #589-#593
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From 0.1 µl to 5000 µl

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Cat. #585-#588 EDVOTEK Fixed Volume MiniPipets™

From 5 µl to 200 µl www.edvotek.com/ Edvotek-Fixed-Volume-MiniPipets





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VISUALIZATION - Equipment



Cat. #558 Midrange UV Transilluminator

Designed to visualize gels stained with either InstaStain® Ethidium Bromide or SYBR® Safe. The UV filter measures 7 x 14 cm. Features a UV blocking cover and an automatic power-cut off when the cover is opened.

www.edvotek.com/558



Cat. #557

TruBlu™ Blue Light Transilluminator

Our NEW TruBlu™ Blue Light Transilluminator is designed to visualize gels stained SYBR® Safe. Viewing area measures 7 x 14 cm. The high intensity control and orange lid ensure superior visualization.

www.edvotek.com/557



Cat. #552 White Light Box

Features a spacious 25 x 25 cm viewing area illuminated by long life LEDs and is housed in a slim aluminum body. It's designed to safely enhance the visualization of gels stained with FlashBlue TM or InstaStain RM Blue.

www.edvotek.com/552

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VISUALIZATION - Stains

SYBR® Safe Stain

Fluoresces with a bright green color when excited with UV light. Engineered to be less mutagenic then Ethidium Bromide, making it much safer to use.

Cat. #608

10,000 X Concentrate for 750 ml. www.edvotek.com/608



InstaStain® Ethidium Bromide

InstaStain® Ethidium Bromide, which contains only a few micrograms of ethidium bromide, is rapid and sensitive. In 2 minutes, an agarose gel is ready for visualization. Disposal of InstaStain® Ethidium Bromide is minimal compared to the volume of liquid waste generated from the standard ethidium bromide staining procedure.

Cat. #2001

For 40 Gels, 7 x 7 cm. www.edvotek.com/2001



Cat. #2002

For 100 Gels, 7 x 7 cm. www.edvotek.com/2002



FlashBlue™ DNA Stain

FlashBlue™ offers simple and rapid staining of agarose gels. FlashBlue™ can be used for both rapid and overnight staining of DNA fragments. No UV light required.

Cat. #609

10x Concentrate for 1.2 L.



www.edvotek.com/609



InstaStain® Blue

InstaStain® Blue sheets stain gels in minutes and give high quality and uniform gel staining with excellent results for photography. They are environmentally friendly, avoiding large amounts of liquid stain and waste disposal.

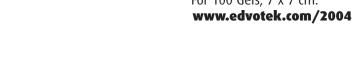
Cat. #2003

For 40 Gels. 7 x 7 cm. www.edvotek.com/2003



Cat. #2004

For 100 Gels, 7 x 7 cm.







Quick Guides: Visualizing DNA

www.edvotek.com/site/pdf/DNA Stain Guide.pdf



Tech Videos: Visualizing DNA

http://bit.ly/stains_playlist



Troubleshooting Guide: PCR & Electrophoresis

PROBLEM:	CAUSE:	ANSWER:
There is very little liquid left in tube after PCR		Make sure the heated lid reaches the appropriate temperature.
	Sample has evaporated	If your thermal cycler does not have a heated lid, overlay the PCR reaction with wax (see Appendix B for details)
ieit iii tube artei i ck		Make sure students close the lid of the PCR tube properly.
	Pipetting error	Make sure students pipet 20 μ L primer mix and 5 μ L Lambda DNA template into the PCR tube.
There's not enough sample in my QuickStrip.	The QuickStrip has dried out.	Add 40 uL water, gently pipet up and down to mix before loading.
	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
The ladder, control DNA, and student PCR products are not visible on the gel.		Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels.
	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.
After staining, the ladder is visible but no PCR	PCR amplification was unsuccessful.	Repeat PCR with fresh PCR EdvoBeads™ and primers.
products are present.		Ensure that the thermal cycler has been properly programmed. See Module II for guidelines.
After staining, the ladder and control PCR products are visible on gel, but some student samples are not present.	Wrong volumes of DNA and primer added to PCR reaction	Practice using pipettes
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™.

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Related Resources

www.edvotek.com/Resources

Here at EDVOTEK®, we've worked hard to bring you some new and exciting resources to make teaching biotechnology easier and more exciting than ever! We've created Lesson Plans and Quick Guide Manuals, FREE for you to download off our website. We have also filmed several Instructional Tech Videos that show step-by-step procedures. We hope you take advantage of these resources and enjoy teaching and learning with EDVOTEK®!



- Using an Adjustable Volume Micropipet
- Preparing Agarose Gels
- · Performing Agarose Gel Electrophoresis
- Diluting a Concentrated Solution
- Visualizing DNA

youtube.com/EdvotekInc

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