EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

EDVO-Kit # 335

Reverse Transcriptase PCR

RT-PCR: A Model for the Molecular Biology of HIV Replication

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is for students to gain an understanding and hands-on experience of the principles and practice of RT-PCR and to relate these reactions to HIV replication.

This experiment is designed for DNA staining with InstaStain® Ethidium Bromide.

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EVT 2010-04-12K

Table of Contents

Components & Requirements

Experiment # 335 contains reagents to perform six sets of RT-PCR reactions.

Sample volumes are very small. For liquid samples, it is important to quick spin the tube contents in a microcentrifuge to obtain sufficient volume for pipeting. Spin samples for 10-20 seconds at maximum speed.

CAUTION!

Wear gloves when handling all tubes for this experiment. RNAse from your fingers will interfere with the experiment results.

		Storage
A.	Tubes with RT-PCR reaction pellets™ Each RT-PCR reaction pellet™ contains Reverse Transcriptase, RNase inhibitor, buffer, nucleotides and *Taq* DNA polymerase	Room Temperature
B.	Primer mix (two primers)	-20°C Freezer
C.	200 base pair ladder	-20°C Freezer
D.	RNAse-free water	-20°C Freezer
E.	RNA Template	-20°C Freezer

Reagents & Supplies

(Store all components below at room temperature)

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- 10x Gel Loading Solution
- InstaStain® Ethidium Bromide
- Microcentrifuge Tubes
- PCR tubes (0.2 ml - for thermal cyclers with 0.2 ml template)
- Calibrated transfer pipets
- Wax beads (for waterbath option or thermal cyclers without heated lid)

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

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

EDVO-Kit # 335

RT-PCR: A Model for the Molecular Biology of HIV Replication

Requirements

- Thermal cycler (EDVOTEK Cat. # 541 highly recommended) or three waterbaths*
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- Water bath (56°C)
- UV Transilluminator or UV Photodocumentation system
- UV safety goggles
- Automatic micropipets (5-50 µl) with tips
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Disposable vinyl or latex laboratory gloves
- Ice buckets and ice
- Distilled or deionized water

*If you do not have a thermal cycler, PCR experiments can be conducted, with proper care, using three waterbaths. However, a thermal cycler assures a significantly higher rate of success.

EDVO-TECH SERVICE

1-800-EDVOTEK
(1-800-338-6835)

Mon - Fri 9 am - 6 pm ET

Technical Service Department

Mon - Fri
9:00 am to 6:00 pm ET

FAX: (301) 340-0582
Web: www.edvotek.com
email: edvotek@aol.com

Please have the following information ready:

- Experiment number and title
- Kit lot number on box or tube
- Literature version number (in lower right corner)
- Approximate purchase date

Human Immunodeficiency Virus

Acquired immune deficiency syndrome (AIDS) is a disease characterized by the progressive deterioration of a patient's immune system. This immunological impairment allows infectious agents such as viruses, bacteria, fungi and parasites to invade the body and propagate. The incidence of certain cancers dramatically increases in these patients because of their compromised immune system. AIDS is a serious threat to human health and is a global problem. Intensive research is being done to advance methods of detection, clinical treatment and prevention.

Retroviruses

The AIDS etiologic agent is the human immunodeficiency virus type 1 (HIV-1), a retrovirus. Retroviruses contain an RNA genome and the RNA-dependent DNA polymerase also termed reverse transcriptase. Members of the retrovirus family are involved in the pathogenesis of certain types of leukemias and other sarcomas in animals. The structure and replication mechanism of HIV is very similar to other retroviruses. HIV is unique in some of its properties since it specifically targets the immune system, is very immunoevasive, forms significant amounts of progeny virus *in vivo* during the later stages of the disease and can also be transmitted during sexual activity.

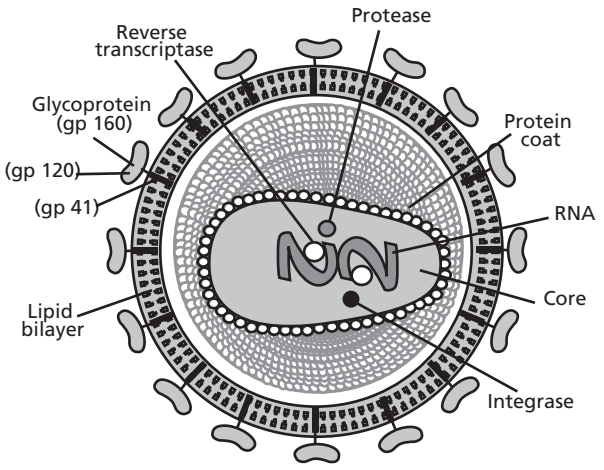


Figure 1 - Overview of HIV structural components

The HIV viral particle is surrounded by a lipid bilayer derived from the host cell membrane during budding (Figure 1). The viral proteins are identified by the prefix gp (glycoprotein) or p (protein) followed by a number indicating the approximate molecular weight in kilodaltons. The lipid bilayer contains gp 160, gp 120 and gp 41. The gp 41 anchors gp 120 in the bilayer. Beneath the bilayer is a capsid consisting of p17 and p18. Within this shell is the viral core. The walls of the core consists of p24 and p25. Within the core are two identical RNA molecules 9000 nucleotides in length. Hydrogen bonded to each genomic RNA is a cellular tRNA molecule. The core also contains reverse transcriptase. Protein products obtained from the HIV genome are displayed in Figure 2.

Large quantities of virus can be grown in tissue culture for diagnostic and research purposes. Several of the viral proteins have been cloned and expressed in relatively large quantities.

Human Immunodeficiency Virus

Background Information

BIOLOGY OF HIV INFECTION

HIV only infects cells which have a $CD4^+$ receptor on their surface. Receptors are used by cells to communicate. They let information in and out of the cell, and different types of cells have different receptors. Two kinds of immune system cells have $CD4^+$ receptors and can be infected by HIV: macrophages (white blood cells called "macs") and $CD4^+$ lymphocytes (also called "TH", "T4 cells" or "$CD4^+$ cells"). In the case of HIV, the viral envelope has protein "spikes" on it, called gp120. These spikes fit the $CD4^+$ receptor on a cell's surface. When the gp120 spike fits onto the host's receptor, it unlocks the receptor and allows HIV to enter the cell. Figure 3 shows HIV binding to a $CD4^+$ cell.

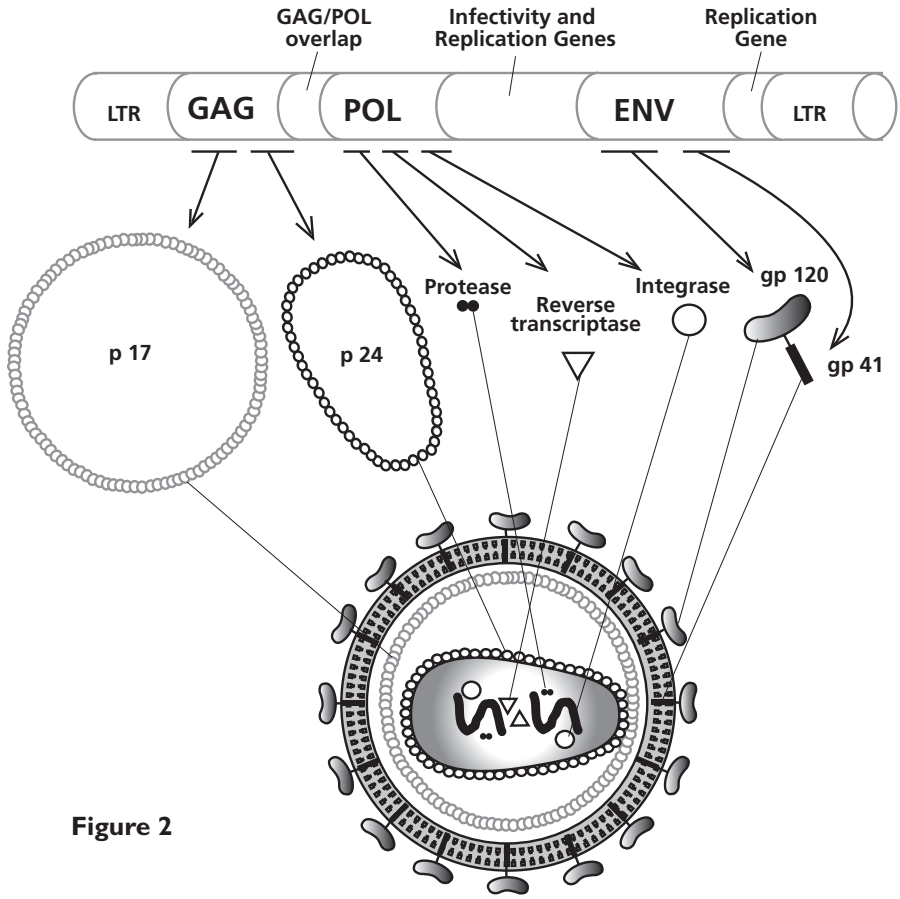


Figure 2

Human Immunodeficiency Virus

Even after HIV binds firmly to the CD4+ receptor, a second molecule, a protein called fusin, is necessary for certain strains of HIV to fuse with the cell membrane and penetrate it. After HIV enters the cell, the process of replication begins with the help of the virus' own enzymes. As described below, reverse transcriptase transcribes the RNA genome of the virus to DNA (provirus). The provirus enters the nucleus of the cell where the viral enzyme integrase inserts it into the host cell's DNA and can synthesize new viral RNA.

Some of this new RNA will become the genetic material contained in new viruses. Some will make the proteins which will coat the new virus core. The HIV proteins will cut the polyproteins into functional sizes. Finally, the viral proteins and the viral RNA are assembled into new HIV and bud off the host cell's surface. Figure 4 shows how HIV attaches to a cell, infects it, and then multiplies.

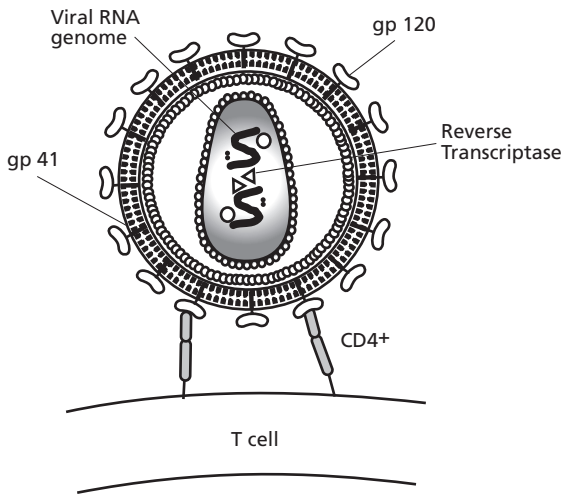


Figure 3

It is important to understand the viral life cycle in order to know how to combat the progression of HIV disease. For example, as researchers learn more about HIV, it is easier to see why treatments with a single drug have failed. HIV is different from other viruses that mutate rapidly because it stays in the body for years, replicating again and again. This large number of rounds of replication multiplied by the high mutation rate makes HIV uniquely dangerous to the host. Researchers continue to examine each stage of the life cycle as well as to note the various ways the immune system fights HIV at different stages. Many drugs called "antiretrovirals" that fight HIV infection are based on different aspects of the structure and function of the HIV molecule.

HIV REPLICATION AND TRANSCRIPTION

Through a complex mechanism involving several events, reverse transcriptase synthesizes a double stranded DNA copy of the genomic RNA template. Transfer RNA acts as the primer for the first DNA strand synthesis, resulting in an RNA-DNA hybrid. RNAse H degrades the RNA strand of the RNA-DNA duplex and the polymerase activity synthesizes a complementary DNA strand.

Background Information

Human Immunodeficiency Virus

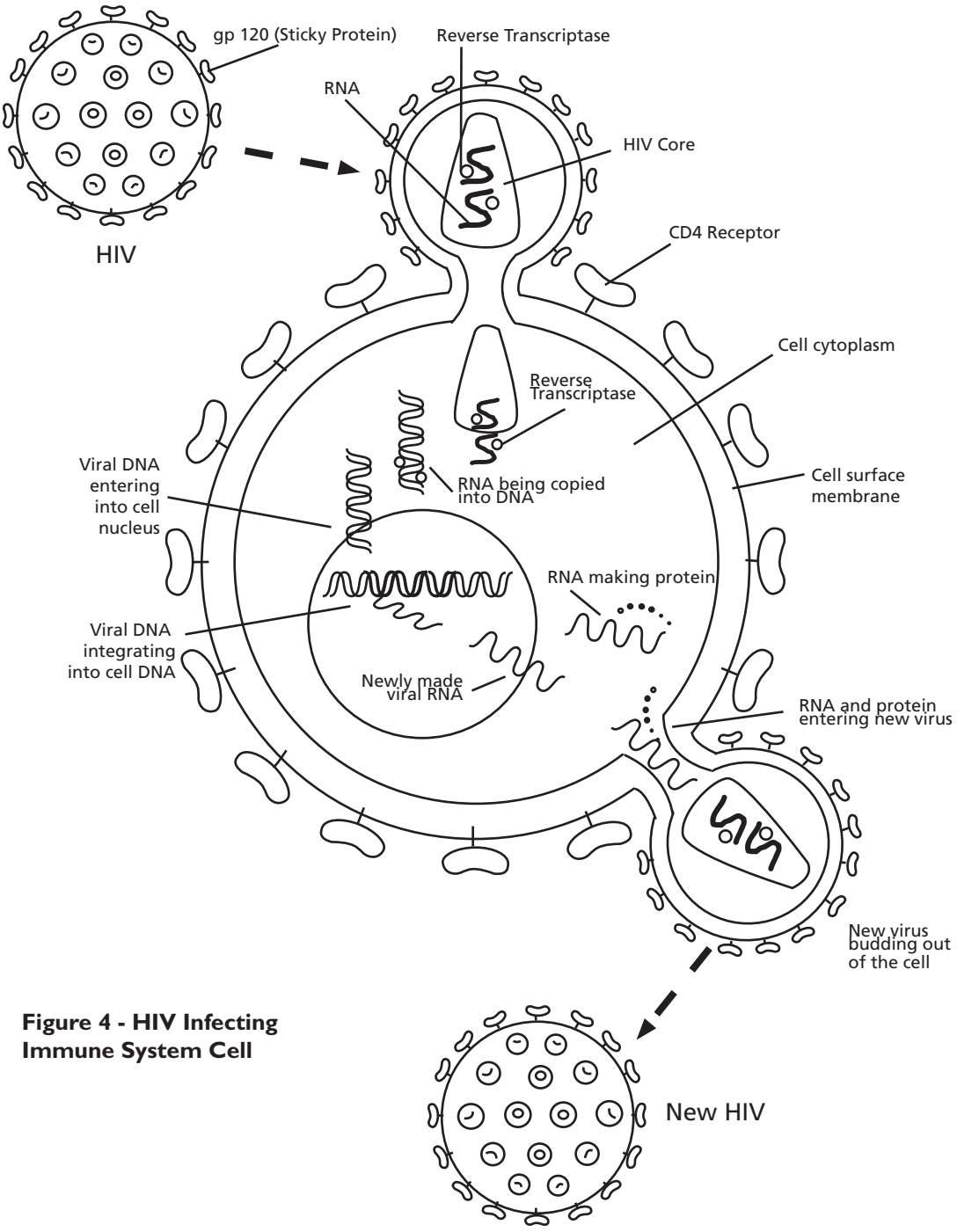


Figure 4 - HIV Infecting Immune System Cell

Human Immunodeficiency Virus

Unlike other cellular DNA polymerases, HIV DNA polymerase (reverse transcriptase) has a high error rate (1 in 10^4). The frequent mutations change the viral protein epitopes. This is believed to be the main mechanism of HIV immunoevasion. The double stranded DNA (dsDNA) migrate into the cell nucleus where they become covalently integrated into the cellular genomic DNA. This integration is catalyzed by HIV integrase.

The viral DNA integrates via specific, self-complementary sequences at both ends called long terminal repeats (LTRs)(see Figure 2). The integrated DNA is called proviral DNA or the provirus. The provirus enters a period of latency that can last for several years. The proviral DNA is replicated along with the cellular DNA and can be inherited through many generations.

The HIV proviral DNA contains the major genes common to all non-transducing retroviruses. These genes are gag, pol and env (see Figure 2). HIV also contains five or six other genes that are much smaller. Retroviral transcription is a complex process producing a variety of RNAs. Production of transcripts is controlled in the LTR and transcriptional termination signals are located in each major gene. The RNA transcripts that remain unspliced become packaged in the new viral particles.

The gag gene is translated into a polypeptide that is cleaved by a viral protease into four proteins that form the inner shells. Specific protease inhibitors are clinically being used to inhibit protein processing and control the further spread of the HIV virus in patients suffering from AIDS. The pol gene encodes the reverse transcriptase and the integrase which is responsible for the genomic incorporation of copy DNA. The env gene encodes the surface glycoproteins the viral particles acquire as they bud from the cells.

cDNA SYNTHESIS AND PCR AMPLIFICATION

There are several ways of obtaining amplified DNA from an RNA template. In one option, cDNA synthesis is performed using reverse transcriptase. A small amount of this product is then transferred to a second reaction tube that contains *Taq* DNA polymerase, buffer and the required reaction substrates for PCR amplification (Figure 5).

In this experiment the two reactions are coupled to reduce sample manipulation and possible contamination. Room temperature RT-PCR pellets are used that contain Reverse Transcriptase, RNase (to digest RNA target), buffer, nucleotides and *Taq* DNA polymerase. In this coupled reaction, reverse transcription occurs first to produce cDNA. This reaction (in the same tube) is followed by the formation of a double stranded DNA followed by exponential PCR amplification (Figure 5).

Human Immunodeficiency Virus

Background Information

Taq polymerase is commonly used in PCR because it remains stable at near-boiling temperatures. Also included in the PCR reaction are the four deoxy-nucleotides (dATP, dCTP, dGTP, and dTTP) and two synthetic oligonucleotides primers, typically 15-30 base pairs in length. These components, together with the DNA to be amplified, are incubated in an appropriate buffer that contains Mg^{+2}. The primers are designed to correspond to the DNA to be amplified also referred to as the target. The PCR reaction mixture is subject-ed to sequential heating/cooling cycles at three different temperatures in a thermal cycler (Figure 5).

- In the first step, the template is heated to near boiling (94° - 96°C.) to denature (melt) the target DNA. This step, known as "denaturation" disrupts the hydrogen bonds between the two complementary DNA strands and causes their separation.

- In the second step, the reaction mixture is cooled to a temperature that is typically in the range of 45° - 65°. In this step, known as "annealing", the two primers that are in great excess to the template, bind to the separated DNA strands.

- In the third step, known as "extension", the temperature is raised to 72°C. At this temperature the *Taq* polymerase is maximally active and adds nucleotides to the two hybridized primers to synthesize the new complementary strands.

RT-PCR reactions are effective as diagnostic tests for HIV infections. Unlike antibody detection tests that require several weeks for a patient to develop detectable amounts of HIV antibodies, PCR based diagnostic tests are based on the presence of the viral particles and will yield results earlier. As a result the course of patient therapy is initiated much earlier.

Human Immunodeficiency Virus

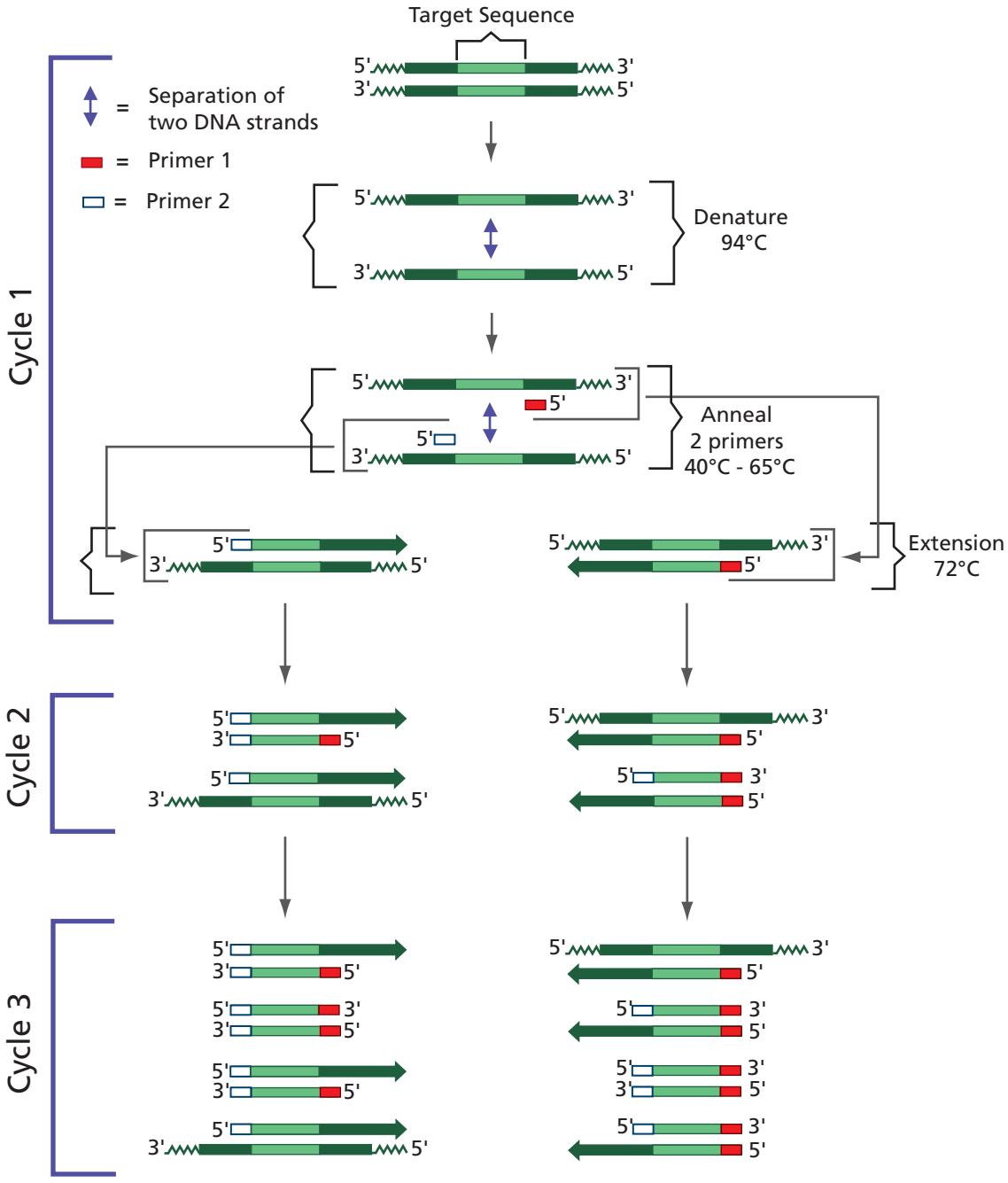


Figure 5: Polymerase Chain Reaction

Experiment Overview and General Instructions

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. If you will be conducting PCR using a thermal cycler without a heated lid, also read the Appendix entitled "Preparation and Handling PCR Samples with Wax ".
3. If you will be using three waterbaths to conduct PCR, read the two appendices entitled "Polymerase Chain Reaction Using Three Waterbaths" and "Handling samples with wax overlays".
4. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT OBJECTIVE:

The objective of this experiment is for students to gain an understanding and hands-on experience of the principles and practice of RT-PCR and to relate these reactions to HIV replication.

BRIEF DESCRIPTION OF EXPERIMENT:

This experiment has three modules:

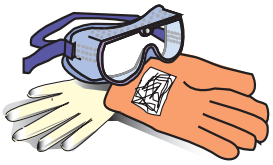
I. Coupled RT-PCR Reaction
II. Separation of RT-PCR Reactions by Electrophoresis
III. Size Determination of RT-PCR Amplified Fragment

GEL SPECIFICATIONS

This experiment requires a gel with the following specifications:

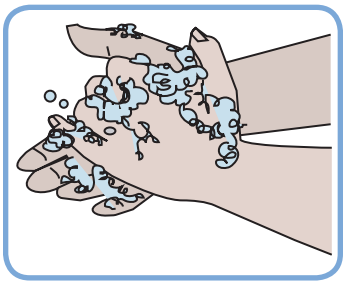
• Recommended gel size	7 x 14 cm (long tray)
• Number of sample wells required	6
• Placement of well-former template	first set of notches
• Gel concentration required	1.0%

Laboratory Safety



Wear gloves and safety goggles

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.
   - Although electrical current from the power source is automatically disrupted when the cover is removed from the apparatus, first turn off the power, then unplug the power source before disconnecting the leads and removing the cover.
   - Turn off power and unplug the equipment when not in use.
5. EDVOTEK injection-molded electrophoresis units do not have glued junctions that can develop potential leaks. However, in the unlikely event that a leak develops in any electrophoresis apparatus you are using, IMMEDIATELY SHUT OFF POWER. Do not use the apparatus.
6. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



Module I - Coupled RT- PCR Reaction

The RT-PCR reaction pellet™ contains Reverse Transcriptase, RNase, buffer, nucleotides and *Taq* DNA polymerase.

Sample volumes are very small. For liquid samples, it is important to quick spin the tube contents in a microcentrifuge to obtain sufficient volume for pipeting. Spin samples for 10-20 seconds at maximum speed.

*The PCR process and *Taq* DNA polymerase are covered by patents owned by Hoffman-La-Roche, Inc.

PCR REACTION:

1. Transfer the RT-PCR Reaction pellet™ to the appropriate sized tube (e.g. 0.5 ml or 0.2 ml) for your thermal cycler.
2. Label the tube containing the RT-PCR reaction pellet™ as "PCR" and with your initials or group number.
3. Tap the reaction tube to assure the RT-PCR reaction pellet is at the bottom of the tube.
4. Add the following to the PCR tube:

B	Primer Mix (two primers)	10 µl
D	RNAse-free Water	30 µl
E	RNA Template for Amplification	10 µl

5. Gently mix the reaction tube.
6. If your thermal cycler is equipped with a heated lid, proceed directly to polymerase chain reaction cycling.

   If your thermal cycler does not have a heated lid, add one wax bead to the tube before proceeding to polymerase chain reaction cycling.

CONTROL REACTION:

7. Label an empty 0.5 ml or 0.2 ml tube (depending on your thermal cycler) "control" and mark it with your initials or group designation. Add to the tube labeled "control" the following:

B	Primer Mix (two primers)	10 µl
D	RNAse Free Water	30 µl
E	RNA Template	10 µl

   Notice that the control tube does not contain the RT-PCR reaction pellet™. What do you think will happen?

8. If your thermal cycler is equipped with a heated lid, proceed directly to polymerase chain reaction cycling.

   If your thermal cycler does not have a heated lid, add one wax bead to the tube before proceeding to polymerase chain reaction cycling.

Module I - Coupled RT- PCR Reaction

POLYMERASE CHAIN REACTION CYCLING

9. Put both PCR tubes through the cycles in a thermal cycler. Preheat the thermal cycler block to 65°C before adding tubes.

10. **Option A:**
If using thermal cycler EDVOTEK Catalog #532, program the thermal cycler according to the following:

1st PCR Program:

Reverse transcription step:	65°C for 40 min.

After the 1st PCR program is complete, proceed to the **2nd PCR Program:**

Denaturation step	94°C for 5 min.
PCR step: 35 cycles	94°C for 30 seconds 50°C for 30 seconds 72°C for 30 seconds
Final extension	72°C for 5 minutes.

Option B:
If using any thermal cycler other than EDVOTEK Catalog #532, program the thermal cycler according to the following:

Reverse transcription step:	65°C for 40 min.
Denaturation step	94°C for 5 min.
PCR step: 35 cycles	94°C for 30 seconds 50°C for 30 seconds 72°C for 30 seconds
Final extension	72°C for 5 minutes.

11. After the cycles are completed, add 5 µl of 10x Gel Loading Solution to the samples and store on ice until ready for electrophoresis.

12. Proceed to instructions for preparing a 1.0% agarose gel (7 x 14 cm) and separating the PCR products by electrophoresis.

OPTIONAL STOPPING POINT

The samples can be held in the thermal cycler at 10°C or frozen after addition of 5 µl of 10x Gel Loading Solution until ready for electrophoresis.

Module II: Separation of PCR Reactions by Agarose Gel Electrophoresis

If you are unfamiliar with agarose gel preparation and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

AGAROSE GEL REQUIREMENTS

- Recommended gel size: 7 x 14 cm

  7 x 14 cm gels are recommended to achieve better resolution of the PCR products. Each gel can be shared by several students or groups.
- Placement of well-former template: first set of notches
- Agarose gel concentration: 1.0%

PREPARING THE AGAROSE GEL

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
2. Place a well-former template (comb) in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.
3. To a 250 ml flask or beaker, add agarose powder and buffer as indicated in the Reference Tables (Appendix A) provided by your instructor. Swirl the mixture to disperse clumps of agarose powder.
4. With a marking pen, indicate the level of the solution volume on the outside of the flask.

Important Note

Continue heating until the final solution appears clear (like water) without any undissolved particles. Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

5. Heat the mixture using a microwave oven or burner to dissolve the agarose powder.
6. Cool the agarose solution to 60°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume marked in step 4.

After the gel is cooled to 60°C:

7. Place the bed on a level surface and pour the cooled agarose solution into the bed.
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.
9. After the gel is solidified, be careful not to damage or tear the wells while removing the rubber dams or tape and comb(s) from the gel bed.
10. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
11. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer (refer to Table B on the instruction Appendix provided by your instructor).

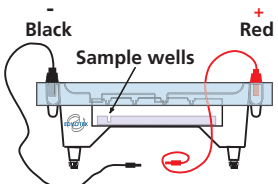
Module II: Separation of PCR Reactions by Agarose Gel Electrophoresis

BEFORE LOADING THE SAMPLES

This experiment requires a 1.0% agarose gel and is designed for staining with InstaStain® Ethidium Bromide.

Reminder:

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



LOADING DNA SAMPLES

1. (Optional Step) Heat the 200 bp DNA ladder and PCR samples for two minutes at 50°C. Allow the samples to cool for a few minutes.

2. Make sure the gel is completely submerged under buffer before loading the samples. Load 25 µl each of the samples in the following sequence.

Lane	Tube	
1	E	200 bp ladder
2	1	Control sample
3	2	RT-PCR sample

3. Record the position of your sample in the gel for easy identification after staining.

RUNNING THE GEL

4. After the DNA samples are loaded, properly orient the cover and carefully snap it onto the electrode terminals.

5. Insert the plugs of the black and red leads into the corresponding inputs of the power source.

6. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor.

7. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.

8. After the electrophoresis is completed, disconnect the power and remove the gel from the bed for staining.

STAINING AND VISUALIZATION OF DNA

After electrophoresis, agarose gels require staining to visualize the separated DNA samples. Your instructor will provide instructions for DNA staining with InstaStain® Ethidium Bromide.

Module III: Size Determination of PCR Amplified DNA Fragment

The Experiment

The size of the RT-PCR amplified fragment can be extrapolated by its migration distance relative to the 200 bp ladder, for which the size of each fragment is known.

1. Measure and record the distance traveled in the agarose gel by each 200 bp fragment starting with the 1000 bp band with appears slightly brighter than the other band. The bands are in 200 bp increments.

   In each case, measure from the lower edge of the sample well to the lower end of each band. Record the distance traveled in centimeters (to the nearest millimeter).

2. Label the semi-log graph paper:

   A. Label the non-logarithmic horizontal x-axis "Migration Distance" in centimeters at equal intervals.

   B. Label the logarithmic vertical y-axis "Log base pairs". Choose your scales so that the data points are well spread out. Assume the first cycle on the y-axis represents 100-1,000 base pairs and the second cycle represents 1,000-10,000 base pairs.

3. For each 200 bp fragment, plot the measured migration distance on the x-axis versus its size in base pairs, on the y-axis.

4. Draw the best average straight line through all the points. The line should have approximately equal numbers of points scattered on each side of the line. Some points may be right on the line.

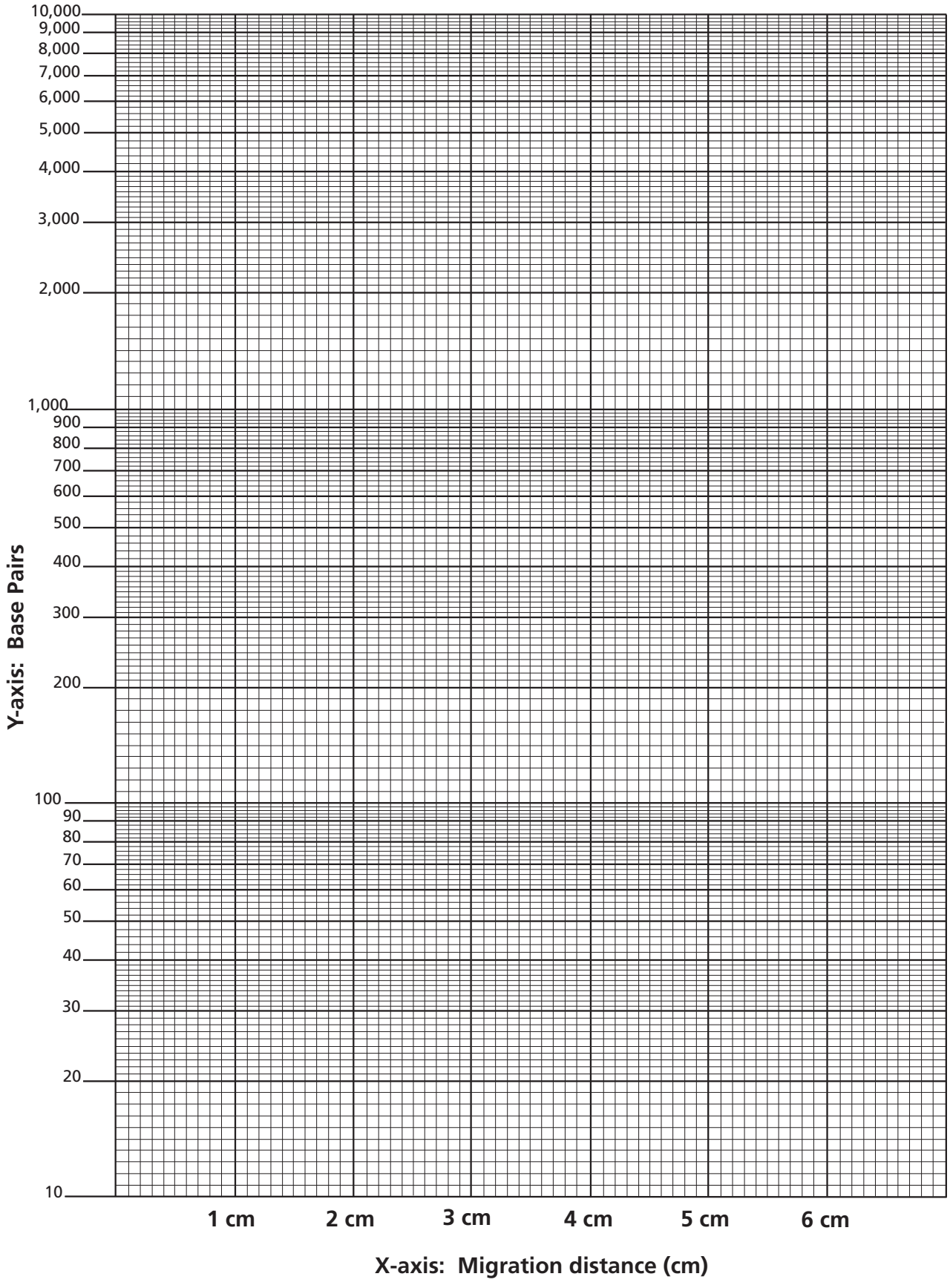
5. Using the graph of the 200 bp ladder, determine the sizes in base pairs of the RT-PCR amplified fragment from this curve.

   A. Find the migration distance of the RT-PCR amplified fragment on the x-axis. Draw a vertical line from that point until the graph line is intersected.

   B. From the point of intersection, draw a second line horizontally to the y-axis and extrapolate the approximate size of the RT-PCR amplified fragment.

Quick Reference:

200 bp fragment sizes: length is expressed in base pairs. The smallest fragment is 200 bp. Next band is 400 bp, 600 bp, etc. The 1000 bp band appears slightly brighter than the other bands.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200
100
90
80
70
60
50
40
30
20
10
Y-axis: Base Pairs
1 cm
2 cm
3 cm
4 cm
5 cm
6 cm
X-axis: Migration distance (cm)

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Why can the onset of AIDS take several years?
2. Why are there so many immunological variants of HIV?
3. What is the source of the four dXTPs required for DNA synthesis?
4. What is the source of the tRNA primer and what is its function in the viral replication?

Instructor's Guide

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

NATIONAL CONTENT AND SKILL STANDARDS

By performing this experiment, students will learn to load samples and run agarose gel electrophoresis. Analysis of the experiments will provide students the means to transform an abstract concept into a concrete explanation. Please visit our website for specific content and skill standards for various experiments.

Technical Service Department

Mon - Fri
9:00 am to 6:00 pm ET

FAX: (301) 340-0582
Web: www.edvotek.com
email: edvotek@aol.com

Please have the following information ready:

- Experiment number and title
- Kit lot number on box or tube
- Literature version number (in lower right corner)
- Approximate purchase date

EDUCATIONAL RESOURCES

Electrophoresis Hints, Help and Frequently Asked Questions

EDVOTEK experiments are easy to perform and designed for maximum success in the classroom setting. However, even the most experienced students and teachers occasionally encounter experimental problems or difficulties. The EDVOTEK web site provides several suggestions and reminders for conducting electrophoresis, as well as answers to frequently asked electrophoresis questions.

Online Ordering now available

Visit our web site for information about EDVOTEK's complete line of "hands-on" experiments for biotechnology and biology education.

Notes to the Instructor:

The Experiment

PCR EXPERIMENTAL SUCCESS GUIDELINES

Please refer to the Appendices section for a summary of important hints and reminders which will help maximize successful implementation of this experiment. This experiment has three modules:

I. Coupled RT-PCR Reaction
II. Separation of RT-PCR Reactions by Electrophoresis
III. Size Determination of RT-PCR Amplified Fragment

MICROPIPETTING BASICS AND PRACTICE GEL LOADING

Accurate pipeting is critical for maximizing successful experiment results. EDVOTEK Series 300 experiments are designed for students who have had previous experience with agarose gel electrophoresis and micropipeting techniques. If your students are unfamiliar with using micropipets, EDVOTEK highly recommends that students perform Experiment # S-44, Micropipetting Basics, or other Series 100 or 200 electrophoresis experiment prior to conducting this advanced level experiment.

APPROXIMATE TIME REQUIREMENTS

1. The RT-PCR cycling will take about 2.0 to 2.5 hours or can be done overnight and the samples held at 10°C.

2. The experiment can be temporarily stopped after the completion of Module I and later resumed. Experimental results will not be compromised if instructions are followed as noted under the heading "Optional Stopping Point" at the end of Module I.

Table C Time and Voltage (1.0% - 7 x 14 cm gel)

Volts	Recommended Time Minimum	Maximum
125	55 min	1 hr 15 min
70	2 hrs 15 min	3 hrs
50	3 hrs 25 min	5 hrs

3. Whether you choose to prepare the gel(s) in advance or have the students prepare their own, allow approximately 30-40 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification. See section "Options for Preparing Agarose Gels" below.

4. The approximate time for electrophoresis will vary from 1 - 5 hours. Generally, the higher the voltage applied, the faster the samples migrate. However, depending upon the apparatus configuration and the distance between the two electrodes, individual electrophoresis units will separate DNA at different rates. Follow manufacturer's recommendations. Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.

Notes to the Instructor:

OPTIONS FOR PREPARING AGAROSE GELS

This experiment is designed for DNA staining after electrophoresis with InstaStain® Ethidium Bromide. There are several options for preparing agarose gels for the experiment.

1. Individual Gel Casting:
   Each student lab group can be responsible for casting their own individual gel prior to conducting the experiment.

2. 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 store gels at -20°C. 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 hot, molten agarose before placing the gels into the apparatus for electrophoresis. This will prevent the gels from sliding around in the trays and the chambers.

3. Batch Gel Preparation:
   A batch of agarose gel can be prepared for sharing by the class. To save time, a larger quantity of UltraSpec-Agarose can be prepared for sharing by the class. See instructions for "Batch Gel Preparation".

GEL CONCENTRATION AND VOLUME

The gel concentration required for this experiment is 1.0%. Prepare gels according to Table A.1 or A.2 in Appendix D.

Notes to the Instructor:

GEL STAINING AND DESTAINING AFTER ELECTROPHORESIS

After electrophoresis, the agarose gels require staining in order to visualize the separated DNA samples. This experiment features a proprietary stain called InstaStain®.

InstaStain® EtBr (Appendix F)

Optimal visualization of PCR products on gels of 1.0% or higher concentration is obtained by staining with InstaStain® Ethidium Bromide (InstaStain® EtBr) cards. Exercise caution when using Ethidium Bromide, which is a listed mutagen. Disposal of the InstaStain® EtBr cards, which contain only a few micrograms of ethidium bromide, is minimal compared to the large volume of liquid waste generated by traditional ethidium bromide staining procedures. Disposal of InstaStain® cards and gels should follow institutional guidelines for chemical waste.

PHOTODOCUMENTATION OF DNA (OPTIONAL)

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.

Notes and Reminders:

- Accurate temperatures and cycle times are critical for PCR. A pre-run for one cycle (approx. 3 to 5 minutes) is recommended to check that the thermal cycler is properly programmed.
- For thermal cyclers that do not have a top heating plate, it is necessary to place a layer of wax above the PCR reactions in the microcentrifuge tubes to prevent evaporation. See Appendix entitled "Preparation and Handling PCR Samples with Wax ".
- Three water baths can be used for PCR if a thermal cycler is unavailable. The experiment will require great care and patience. Samples will require wax layers. See appendices entitled "Polymerase Chain Reaction Using Three Waterbaths" and "Handling samples with wax overlays".

Pre-Lab Preparations

MODULE I - PCR REACTION

1. **Option A:**
   If using thermal cycler EDVOTEK Catalog #532, program the thermal cycler according to the following:

   1st PCR Program:

Reverse transcription step:	65°C for 40 min.

   After the 1st PCR program is complete, proceed to the **2nd PCR Program:**

Denaturation step	94°C for 5 min.
PCR step: 35 cycles	94°C for 30 seconds 50°C for 30 seconds 72°C for 30 seconds
Final extension	72°C for 5 minutes.

   Option B:
   If using any thermal cycler other than EDVOTEK Catalog #532, program the thermal cycler according to the following:

Reverse transcription step:	65°C for 40 min.
Denaturation step	94°C for 5 min.
PCR step: 35 cycles	94°C for 30 seconds 50°C for 30 seconds 72°C for 30 seconds
Final extension	72°C for 5 minutes.

Each Group Requires:

A	One RT-PCR Reaction pellet* in tube	
B	Primer Mix	22 µl
C	200 bp ladder	25 µl
D	RNA-free Water	100 µl
E	RNA Template	22 µl
F	10x Gel Load	50 µl
•	One 0.5 ml tube for control reaction	

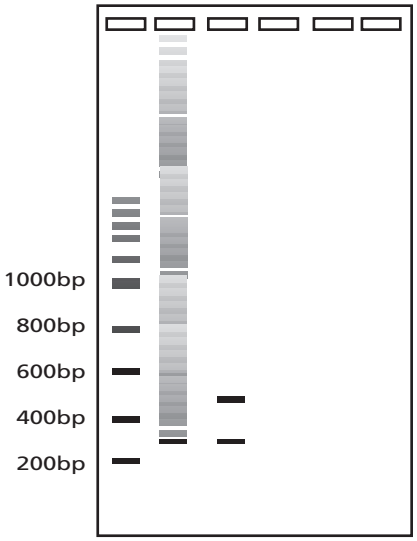
2. Wear gloves when handling all tubes for this experiment. RNA from your fingers will interfere with the experimental results.

3. Place components B and E on ice. Aliquot reagents for student groups using a fresh pipet tip for each component, or set up a pipeting station for the class. Each group requires one of the RT-PCR pellets.

* The RT-PCR reaction pellet contains Reverse Transcriptase, RNase inhibitor, buffer, nucleotides and *Taq* DNA polymerase.

Experiment Results and Analysis

Idealized Schematic



The gel photo example shows the approximate relative amplifications of the PCR amplified band. Smaller fragments stain less efficiently and will appear as fainter bands.

Lane 1 200 bp ladder

Lane 2 Control Total RNA appears as a smear. (sample contains RNA template, primer mix and water)

Lane 3 RT-PCR reaction sample.

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

Appendices

A PCR Experimental Success Guidelines
B Polymerase Chain Reaction Using Three Waterbaths
C Preparation and Handling of PCR Samples With Wax
D 1.0% Agarose Gel Preparation
E 1.0% Agarose Gels - Quantity Preparations
F Staining and Visualization of DNA with InstaStain® Ethidium Bromide Cards

- Material Safety Data Sheets

EDVO-Kit # 335

Appendix A

PCR Experimental Success Guidelines

EDVOTEK experiments which involve the amplification of DNA are extremely relevant, exciting and stimulating classroom laboratory activities. These experiments have been performed successfully in many classrooms across the country, but do require careful execution because of the small volumes used. The following guidelines offer some important suggestions, reminders and hints for maximizing success.

THE PCR REACTION

1. **Add Primers and DNA to the RT-PCR Reaction pellet:** Add the primer mixture (forward and reverse primers) and the cell DNA (supernatant) as specified in the experimental procedures to the microcentrifuge tube containing the RT-PCR reaction pellet. Make sure that the pellet (which contains the Reverse Transcriptase, RNase, buffer, nucleotides and *Taq* DNA polymerase) is completely dissolved. Do a quick spin in a microcentrifuge to bring the entire sample to the bottom of the tube. Prepare the control reaction similarly.

2. **The Thermal cycler:** It is critical that the thermal cycler be accurately programmed for the correct cycle sequence, temperatures and the time for each of the cycles.

3. **Oil or Wax:** For thermal cyclers that do not have a top heating plate, the reaction in the tubes must be overlaid with oil or wax to prevent evaporation.

4. **Manual Water Bath PCR:** Three water baths can be used as an alternative to a thermal cycler for PCR, but results are more variable. Samples require oil or wax layers. This method requires extra care and patience.

GEL PREPARATION AND STAINING

5. **Concentrated agarose:** Gels of higher concentration (> 0.8%) require special attention when dissolving or re-melting. Make sure that the solution is completely clear of "clumps" or glassy granules. Distorted electrophoresis DNA band patterns will result if the gel is not properly prepared.

6. **Electrophoretic separation:** The tracking dye should travel at least 6 cm from the wells for adequate separation before staining.

7. **Staining:** Staining of higher concentration gels (> 0.8%) require special care to obtain clear, visible results. After staining (15 to 30 min.) with InstaStain® Ethidium Bromide or liquid ethidium bromide, examine the results using a UV (300 nm) transilluminator. Repeat the staining as required.

8. **DNA 200 bp ladder:** After staining the agarose gel, the DNA 200 bp ladder (markers) should be visible. If bands are visible in the markers and control lanes, but bands in the sample lanes are faint or absent, it is possible that DNA was not successfully extracted from the cells. If the ladder, control and DNA bands are all faint or absent, potential problems could include improper gel preparation, absence of buffer in the gel, improper gel staining or a dysfunctional electrophoresis unit or power source.

Appendix B

EDVO-Kit # 335

Polymerase Chain Reaction Using Three Waterbaths

Superior PCR results are obtained using an automated thermal cycler. However, if you do not have a thermal cycler, this experiment can be adapted to use three waterbaths (Cat. # 544). Much more care needs to be taken when using the three-waterbath PCR method. The PCR incubation sample is small and can easily be evaporated. Results using three waterbaths are often variable. **Please refer to the Appendix entitled "PCR Samples with Wax Overlays" for sample handling and preparation tips.**

PREPARATION OF THE PCR REACTION:

1. The PCR reaction sample should be prepared as specified in the experiment instructions. Each PCR reaction sample contains three critical components:
   - PCR Reaction pellet™
   - Primer mix
   - DNA for amplification

Each PCR Reaction pellet contains *Taq* DNA polymerase, four deoxytriphosphates, Mg^{+2} and buffer.

2. After adding the components of the PCR reaction sample, use clean forceps to transfer one wax bead to the PCR tube. At the start of the PCR reaction, the wax will melt and overlay the samples to prevent evaporation during heating.

POLYMERASE CHAIN REACTION CYCLING

3. In the three-waterbath PCR method, the PCR reaction sample is sequentially cycled between three separate waterbaths, each set at different temperatures, for a specified period of time. The sequential placement of the reaction sample in the waterbaths maintained at three different temperatures constitutes one PCR cycle. One example of a PCR cycle might be as follows:

   94°C for 1 minute
   50°C for 1 minute
   72°C for 1 minute

   See experiment instructions for specific program requirements.

Important Note

It is imperative that temperatures are accurately maintained throughout the experiment.

4. The PCR tube must be handled carefully when sequentially cycled between the three waterbaths. For each cycle:
   - Carefully place the PCR tube in a waterbath float. Make sure that the sample volume is at the bottom of the tube and remains undisturbed. If necessary, pulse spin the tube in a balanced microcentrifuge, or shake the tube to get all of the sample to the bottom of the tube.
   - Use forceps to carefully lower the waterbath float (with tubes) sequentially into the waterbaths.
5. Process the PCR reaction sample for the total number of cycles specified in the experiment instructions. On the final cycle the 72°C incubation can be extended to 5 minutes.
6. After all the cycles are completed, the PCR sample is prepared for electrophoresis.

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Appendix C

Preparation and Handling of PCR Samples With Wax

For Thermal Cyclers without Heated Lids, or PCR Using Three Waterbaths

Automated thermal cyclers with heated lids are designed to surround the entire sample tube at the appropriate temperature during PCR cycles. Heating the top of the tubes during these cycles prevents the very small sample volumes from evaporating. For thermal cyclers without heated lids, or when conducting PCR by the three-waterbath method, it is necessary to add a wax bead to the reaction sample. During the PCR process, the wax will melt and overlay the samples to prevent evaporation during heating.

PREPARING THE PCR REACTION:

1. The RT-PCR reaction sample should be prepared as specified in the experiment instructions. Each RT-PCR reaction sample contains the following three critical components:

   - RT-PCR Reaction pellet™
   - Primer mix
   - DNA for amplification

Each RT-PCR Reaction pellet contains Reverse Transcriptase, RNase, buffer, nucleotides and *Taq* DNA polymerase.

2. After adding the components of the RT-PCR reaction sample, use clean forceps to transfer one wax bead to the PCR tube.

3. Process the RT-PCR reaction sample for the total number of cycles specified in the experiment instructions.

PREPARING THE PCR REACTION FOR ELECTROPHPORESIS:

4. After the cycles are completed, transfer the PCR tube to a rack and prepare the PCR sample for electrophoresis.

   - Place the PCR tube in a 94°C waterbath long enough to melt the wax overlay. Use a clean pipet to remove most of the melted wax overlay.
   - Allow a thin layer of the wax to solidify.
   - Use a clean pipet tip to gently poke a hole through the solidified wax. Remove the tip.
   - Use another clean pipet tip to enter the hole to remove the volume of mixture specified in the experiment instructions. Transfer this volume to a clean tube.
   - Add other reagents according to experiment instructions, if applicable,.
   - Add 5 µl of 10x Gel Loading solution to the sample and store on ice.

5. Proceed to delivery of the sample onto an agarose gel for electrophoresis as specified in the experiment instructions.

Appendix D

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1.0% Agarose Gel Preparation

If preparing the gel with concentrated (50x) buffer, use Table A.1.

If preparing the gel with diluted (1x) buffer, use Table A.2.

Table A.1 Individual 1.0% UltraSpec-Agarose™ Gel

Size of Gel (cm)	Amt of Agarose (g)	+ Concentrated Buffer (50X) (ml)	+ Distilled Water (ml)	= **Total Volume (ml)**
7 x 7	0.25	0.5	24.5	**25**
7 x 14	0.5	1.0	49.0	**50**

Table A.2 Individual 1.0% UltraSpec-Agarose™ Gel

Size of Gel (cm)	Amt of Agarose (g)	+ Diluted Buffer (1x) (ml)
7 x 7	0.25	25
7 x 14	0.5	50

For DNA analysis, the recommended electrophoresis buffer is Tris-acetate-EDTA, pH 7.8. The formula for diluting EDVOTEK (50x) concentrated buffer is one volume of buffer concentrate to every 49 volumes of distilled or deionized water. Prepare buffer as required for your electrophoresis unit.

Table B Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Total Volume Required (ml)	Dilution: 50x Conc. Buffer (ml)	+ Distilled Water (ml)
M6+	300	6	294
M12	400	8	392
M36 (blue)	500	10	490
M36 (clear)	1000	20	980

Table C Time and Voltage (1.0% - 7 x 14 cm gel)

Volts	Recommended Time: Minimum	Maximum
125	55 min	1 hr 15 min
70	2 hrs 15 min	3 hrs
50	3 hrs 25 min	5 hrs

Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C. The approximate time for electrophoresis will vary from approximately 1 - 5 hours depending upon various factors. Conduct electrophoresis for the length of time determined by your instructor.

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Appendix E

1.0% Agarose Gels - Quantity Preparations

To save time, 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 can be remelted.

Table D

Bulk Preparation of Electrophoresis Buffer

Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
60		2,940		3000 (3 L)

Table E

Batch Preparation of 1.0% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
3.0		6.0		294		300
4.0		8.0		392		400

Note: The UltraSpec-Agarose™ kit component is often 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.

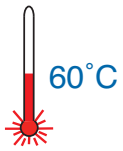
BULK ELECTROPHORESIS BUFFER

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

BATCH AGAROSE GELS (1.0%)

For quantity (batch) preparation of 1.0% agarose gels, see Table E.

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour appropriate amount 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. The volume required is dependent upon the size of the gel bed.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

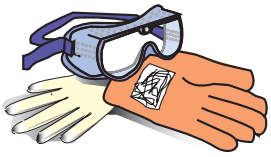
Appendix F

EDVO-Kit # 335

Staining and Visualization of DNA

INSTASTAIN® ETHIDIUM BROMIDE CARDS

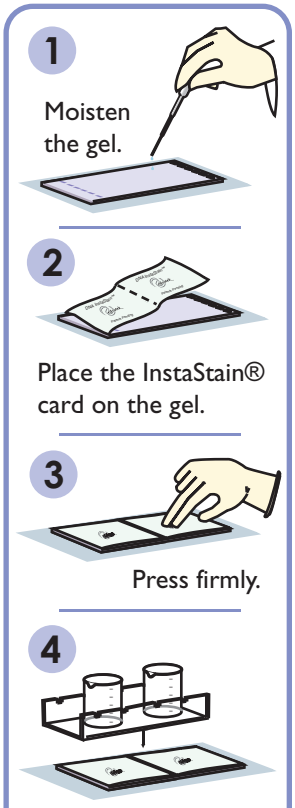
Do not stain gel(s) in the electrophoresis apparatus.



Wear gloves and safety goggles

1. After electrophoresis, place the gel on a piece of plastic wrap on a flat surface. Moisten the gel with a few drops of electrophoresis buffer.
2. Wearing gloves, remove the clear plastic protective sheet, and place the unprinted side of the InstaStain® EtBr card on the gel.
3. Firmly run your fingers over the entire surface of the InstaStain® EtBr. Do this several times.
4. Place the gel casting tray and a small empty beaker on top to ensure that the InstaStain® card maintains direct contact with the gel surface.

   Allow the InstaStain® EtBr card to stain the gel for 10-15 minutes.
5. After 10-15 minutes, remove the InstaStain® EtBr card. Transfer the gel to a ultraviolet (300 nm) transilluminator for viewing. Be sure to wear UV protective goggles.



Visit our web site for an animated demonstration of InstaStain® EtBr.

www.edvotek.com

Caution: Ethidium Bromide is a listed mutagen.

Disposal of InstaStain

Disposal of InstaStain® cards and gels should follow institutional guidelines for chemical waste.

Additional Notes About Staining

- If bands appear faint, or if you are not using EDVOTEK UltraSpec-Agarose™, gels may take longer to stain with InstaStain® EtBr. Repeat staining and increase the staining time an additional 10-15 minutes.
- DNA 200 bp markers should be visible after staining even if the amplified DNA samples are faint or absent. If markers are not visible, troubleshoot for problems with the electrophoretic separation.

EDVO-Kit # 335

Material Safety Data Sheets

Full size (8.5 x 11") pdf copy of MSDS available at www.edvotek.com or by request.

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Agarose

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS #9012-36-6				

Section III - Physical/Chemical Characteristics

Boiling Point: For 1% solution 194 F		Specific Gravity (H 0 = 1): 2	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Insoluble - cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D	N.D.

Extinguishing Media: Water spray, dry chemical, carbon dioxide, halon or standard foam

Special Fire Fighting Procedures: Possible fire hazard when exposed to heat or flame

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability		Conditions to Avoid
Unstable		None
Stable	X	

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

Hazardous Polymerization		Conditions to Avoid
May Occur		None
Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Inhalation: No data available Ingestion: Large amounts may cause diarrhea

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Sweep up and place in suitable container for disposal

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece.

Ventilation		Special
Local Exhaust		
Mechanical	Gen. dilution ventilation	Other

Protective Gloves: Yes Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Impervious clothing to prevent skin contact

Work/Hygienic Practices: None

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): 50x Electrophoresis Buffer

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity ($H_2$0 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Appreciable, (greater than 10%)

Appearance and Odor: Clear, liquid, slight vinegar odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D.	N.D.

Extinguishing Media: Use extinguishing media appropriate for surrounding fire.

Special Fire Fighting Procedures: Wear protective equipment and SCBA with full facepiece operated in positive pressure mode.

Unusual Fire and Explosion Hazards: None identified

Section V - Reactivity Data

Stability		Conditions to Avoid
Unstable		None
Stable	X	

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

Hazardous Polymerization		Conditions to Avoid
May Occur		None
Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): None

Carcinogenicity: None identified NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Irritation to upper respiratory tract, skin, eyes

Medical Conditions Generally Aggravated by Exposure: None

Emergency First Aid Procedures: Ingestion: If conscious, give large amounts of water

Eyes: Flush with water Inhalation: Move to fresh air Skin: Wash with soap and water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing. Mop up spill and rinse with water, or collect in absorptive material and dispose of the absorptive material.

Waste Disposal Method: Dispose in accordance with all applicable federal, state, and local enviromental regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation		Special
Local Exhaust	Yes	None
Mechanical (General)	Yes	None

Protective Gloves: Yes Eye Protection: Safety goggles

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Gel loading solution concentrate, 10x

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/10/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity ($H_2$0 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: soluble

Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data	No data	No data	

Extinguishing Media: Dry chemical, carbon dioxide, water spray or foam

Special Fire Fighting Procedures: Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.

Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability		Conditions to Avoid
Unstable		None
Stable	X	

Incompatibility: None known

Hazardous Decomposition or Byproducts: Sulfur oxides and bromides

Hazardous Polymerization		Conditions to Avoid
May Occur		None
Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute eye contact: May cause irritation No data available for other routes

Carcinogenicity: None NTP? No data IARC Monographs? No data OSHA Regulation? No data

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Rinse contacted area with copious amounts of water.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with organic vapor cartridge.

Ventilation		Special
Local Exhaust	Yes	Yes
Mechanical (General)	Yes	Other None

Protective Gloves: yes Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): InstaStain® Ethidium Bromide

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name
InstaStain, Inc.
P.O. Box 1232
West Bethesda, MD 20827

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Ethidium Bromide		Data not available		
(2,7-Diamino-10-Ethyl-9-Phenylphenanthridinium Bromide)				
CAS# 139-33-3				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D.	N.D.

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam

Special Fire Fighting Procedures: Wear protective clothing and SCBA to prevent contact with skin & eyes

Unusual Fire and Explosion Hazards: Emits toxic fumes

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide, nitrogen oxides, hydrogen bromide gas

Hazardous Polymerization	May Occur		Conditions to Avoid: None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Chronic: May alter genetic material
Acute: Material irritating to mucous membranes, upper respiratory tract, eyes, skin

Carcinogenicity: No data available NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Irritation to mucous membranes and upper respiratory tract

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear SCBA, rubber boots, rubber gloves

Waste Disposal Method: Mix material with combustible solvent and burn in a chemical incinerator equipped afterburner and scrubber

Precautions to be Taken in Handling and Storing: Use in chemical fume hood with proper protective lab gear.

Other Precautions: Mutagen

Section VIII - Control Measures

Respiratory Protection (Specify Type): SCBA

Ventilation	Local Exhaust: Yes	Special: Chem. fume hood	
	Mechanical (General): No	Other: None	

Protective Gloves: Rubber	Eye Protection: Chem. safety goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Use in chemical fume hood with proper protective lab gear.

EDVO-Kit # 335

Material Safety Data Sheets

Full size (8.5 x 11") pdf copy of MSDS available at www.edvotek.com or by request.