Experiment Objective:

The objective of this experiment is to introduce the principles of extracting plasmid DNA from bacterial cells. Students will develop an understanding of the structure and function of plasmid DNAs.

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 contains reagents for 20 plasmid isolations (Mini-preps) and enough electrophoresis reagents to prepare and run five agarose gels based upon the use of Horizontal gel electrophoresis apparatus, Model #M12.

### Contents

- A  Tris Buffer concentrate
- B  Sodium Hydroxide solution
- C  SDS solution (Sodium dodecyl sulfate, 10%)
- D  Potassium acetate neutralization buffer
- E  RNase (DNase-free)

- Plasmid Extraction LyphoCells™ (freeze-dried)
- 10x Gel Loading Solution
- Practice Gel Loading Solution
- UltraSpec-Agarose™ powder (2.5 g.)
- 50x concentrated electrophoresis buffer
- DNA Blue InstaStain™ sheets
- 10x concentrated Methylene Blue Plus™ stain
- 1 ml pipets
- 100 ml plastic graduated cylinder
- Microcentrifuge tubes
- Microtipped Transfer Pipets
Experiment Requirements

PLASMID ISOLATION

- Microcentrifuge
- Water bath (37°C)
- Automatic micropipets with tips
- Pipet pumps
- 95-100% isopropanol
- Ice

AGAROSE GEL ELECTROPHORESIS

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Hot plate, Bunsen burner or microwave oven
- Recommended equipment:
  - DNA visualization system (white light)
  - Staining Tray and Net
- 5 or 10 ml pipets
- Pipet pumps
- 250 ml beakers or flasks
- Hot gloves
- Marking pens
- Distilled or deionized water
Experiment at a Glance

EXPERIMENT #202
ISOLATION OF PLASMID DNA

PROTOCOL

OPTION 1
Procedures for Plasmid Isolation start on page 9

AGAROSE GEL ELECTROPHORESIS
Experimental Procedures Page 13

DNA STAINING, VISUALIZATION AND ANALYSIS
Page 17

OPTION 2
Requires Cat. # 622 Deproteinization Matrix

Procedures for Plasmid Isolation start on page 11

RESTRICTION ENZYME DIGESTION
Requires Restriction Enzymes, not included with experiment.

AGAROSE GEL ELECTROPHORESIS
Experimental Procedures Page 13

DNA STAINING, VISUALIZATION AND ANALYSIS
Page 17
Isolation of Plasmid DNA

Many types of bacteria contain plasmid DNA. Plasmids are extrachromosomal, double-stranded circular DNA molecules generally containing 1,000 to 100,000 base pairs. Even the largest plasmids are considerably smaller than the chromosomal DNA of the bacterium, which can contain several million base pairs. Certain plasmids replicate independently of the chromosomal DNA and can be present in hundreds of copies per cell. A wide variety of genes have been discovered in plasmids. Some of them code for antibiotic resistance and restriction enzymes. Plasmids are extremely important tools in molecular cloning because they are useful in propagating foreign genes. When plasmids are used for these purposes, they are referred to as vectors.

Through the use of recombinant DNA technology, hundreds of artificial vectors have been constructed from elements of naturally occurring plasmids. These vectors have specifically designed properties that make them useful in solving particular experimental problems. For example, synthetic oligodeoxynucleotide linkers have been incorporated into many plasmid vectors. These linkers contain many different restriction enzyme recognition sites to facilitate the insertion of foreign DNA. The linkers are often placed near characteristic marker genes or high efficiency transcriptional promoters, both of which aid in the isolation and expression of the cloned DNA.

Plasmid DNA naturally exists as a supercoiled molecule. Supercoiling arises from alterations in the winding of the two DNA strands around each other. In certain areas of the molecule, the DNA strands are wound around each other less frequently than in non-supercoiled DNA. The strain caused by these alterations...
Isolation of Plasmid DNA, continued

create deformations in the DNA. These deformations partially relieve the strain and ultimately lead to supercoiling. Supercoiled DNA is folded onto itself and has a more condensed and entangled structure than the same DNA which is relaxed. As an analogy to supercoiling, consider a rubber band. When the rubber band is twisted, it eventually becomes knotted and collapses onto itself as an entangled ball.

Purified DNA must be a covalently closed circle to exist as a supercoiled molecule. Supercoiled plasmid DNA is often called Form I DNA. Supercoiling in the cell is caused by the action of enzymes called DNA gyrases. These enzymes use the chemical energy in ATP to introduce supercoiling into a relaxed molecule. In addition, there are enzymes that relax supercoiled DNA and are called unwinding or relaxing enzymes. Supercoiling has important biological consequences. Very large DNA molecules would simply not fit in the cell if they were not supercoiled. Gene expression can also be influenced by supercoiling.

If one or more phosphate bonds anywhere in the backbone of supercoiled DNA are broken, the molecule unravels to a relaxed form called open circular DNA or Form II DNA. These breaks in the phosphate backbone are called nicks. Nicked double-stranded DNA is not covalently closed. The two strands of nicked DNA are still held together by hydrogen bonds between the bases. With time, purified supercoiled DNA slowly develops nicks and converts to Form II. This is because supercoiled DNA is not as stable as its relaxed or open circular forms. Endonucleases, such as DNAse I, will randomly nick supercoiled DNA when used in low amounts. Nicking can also be introduced by mechanical manipulations during plasmid purification.

During replication, several of the same plasmid molecules can form interlocked rings. These multimers of plasmid are called catenanes. A catenane containing two of the same plasmid molecules is called a dimer. Similarly, those containing three or four molecules are called trimers and tetramers, respectively. Each plasmid molecule in a catenane can be supercoiled, however, for clarity, they are represented as relaxed circles.
BACKGROUND INFORMATION

Isolation of Plasmid DNA, continued

Agarose gel electrophoresis is a powerful separation method frequently used to analyze plasmid DNA. The agarose gel consists of microscopic pores that act as a molecular sieve. Samples of DNA can be loaded into wells made in the gel during molding. When an electric field is applied, the DNA molecules are separated by the pores in the gel according to their size and shape. Generally, smaller molecules pass through the pores more easily than larger ones. Since DNA has a strong negative charge at neutral pH, it will migrate towards the positive electrode in the electrophoresis apparatus. The rate at which a given DNA molecule migrates through the gel depends not only on its size and shape, but also on the type of electrophoresis buffer, the gel concentration and the applied voltage. Under the conditions that will be used for this experiment, the different forms of the same plasmid DNA molecule have the following rates of migration (in decreasing order):

Supercoiled > linear > Nicked Circles >
dimer > trimer > etc.

Supercoiled DNA has the fastest migration rate of the different forms of plasmid. In the plasmid extraction experiment you will be doing, there will be some residual, degraded RNA which consists of transfer RNA and digested ribosomal and messenger RNA. Degraded RNA has a faster migration rate than supercoiled plasmid DNA because it is much smaller in size.

In the first step of the experiment a cell lysis solution is added to the cells. This solution contains the detergent sodium dodecyl sulfate (SDS) which dissolves the cell membrane and denatures proteins. The solution is very alkaline (pH > 12) due to the presence of sodium hydroxide. The high pH aids in denaturing proteins and causes the cleavage of the phosphate bonds in RNA. This eliminates interference from high molecular weight RNA during the plasmid purification. Under highly alkaline conditions, the two strands in non-supercoiled DNA (linear fragments of chromosomal DNA, relaxed and nicked circular DNA) separate and are partially removed from solution. However, this does not occur with supercoiled forms of plasmid DNA because the two strands are intertwined and entangled in a way that prevents them from coming apart. Therefore, supercoiled plasmid remains free in solution.

The potassium acetate neutralization buffer contains acetic acid and potassium salts. The acidic buffer neutralizes the alkaline conditions created by the sodium hydroxide. The potassium causes the SDS, with its associated membrane fragments and proteins, to precipitate. The chromosomal DNA of E. coli is attached at several points to the cell membrane. Centrifugation of the potassium-SDS-membrane complexes also removes large amounts of entrapped chromosomal DNA.
Isolation of Plasmid DNA, continued

The addition of isopropanol precipitates the plasmid and remaining RNA. Tris buffer (diluted buffer concentrate for RNase) is used to re-suspend the DNA precipitate in a higher concentration. The buffer contains the enzyme RNase, which further degrades RNA. The concentrated gel loading solution prepares the sample for electrophoresis by making it denser than the electrophoresis buffer. This enables the sample to sink into the wells of the submerged gel. A negatively charged, blue tracking dye is also present to monitor the electrophoresis and to make sample loading easier.

In this experiment, a 3000 base pair plasmid will be extracted from E. coli cells. The restriction map for this plasmid has a single site for Eco RI. Digestion with the enzyme will yield a single band measuring 3,000 ± 300 nucleotides. The multiple forms of the plasmid will be converted to the linear form.
**EXPERIMENTAL PROCEDURES**

Option 1 - Isolation of Plasmid DNA for Detection on Gels

**EXPERIMENT OBJECTIVE:**

The objective of this experiment is to introduce the principles of extracting plasmid DNA from bacterial cells. Students will develop an understanding of the structure and function of plasmid DNAs.

**LABORATORY SAFETY**

This experiment is designed for staining of DNA with either DNA Blue InstaStain™ or Methylene Blue Plus™ stain after electrophoresis. As with any biological stain, care should be taken when handling solutions or gels containing methylene blue. Gloves and goggles should be worn when handling staining reagents, and worn routinely throughout the experiment as good laboratory practice.

**ISOLATION OF PLASMID DNA FOR DETECTION ON GELS**

1. Obtain a microcentrifuge tube of suspended *E. coli* cells and put your initials or group number on it. Place the tube on ice.

2. Using a designated 1 ml pipet, add 0.4 ml of Cell Lysis Solution (contains sodium hydroxide and SDS). This step will denature the proteins.

3. Cap the tube and gently mix by inverting the tube 5 times.

4. Keep on ice for 5 minutes.

5. Using a designated 1 ml pipet, add 0.3 ml of potassium acetate neutralization buffer (D). Potassium salt of SDS will precipitate from solution in the cold. This step will remove the SDS.

6. Cap the tube and mix thoroughly by inverting the tube. A white precipitate should form.

7. Keep the tube on ice for 4 minutes.

8. Place the tube in a microcentrifuge with a counterbalance. (Another group’s tube will serve this purpose, or a tube containing 0.7ml of water.)

9. Centrifuge at full speed (10,000 to 14,000 rpm) for 5 minutes.

**Important Note:**

Plasmid preparations from this procedure will not be effectively digested with restriction enzymes. Additional deproteinization is necessary, and can be accomplished using EDVOTEK Cat. # 622, Deproteinization Matrix. Follow the Module 2 experimental procedures.

**Remember!**

Place the 95-100% Ethanol or Isopropanol on ice before the lab starts.

Allow adequate time to equilibrate a water bath at 37°C for step 21.

Use a fresh pipet when going into different stock solutions to avoid cross contamination.
Isolation of Plasmid DNA for Detection on Gels, continued

**Useful Hint!**

**Step 10:**
After centrifugation, the precipitate will appear as a fluffy material adhering to the wall of the microcentrifuge tube.

Try to avoid touching the inside wall of the tube when transferring the supernatant into a fresh microcentrifuge tube.

**Step 16:**
Be careful not to dislodge the pellet and aspirate it into the transfer pipet.

**Remember!**

**Step 10:**
After centrifugation, the precipitate will appear as a fluffy material adhering to the wall of the microcentrifuge tube.

Try to avoid touching the inside wall of the tube when transferring the supernatant into a fresh microcentrifuge tube.

10. When centrifugation is finished, pipet 0.5 ml of the supernatant (which contains plasmid DNA) into a fresh microcentrifuge tube.

   Try to avoid transferring any of the precipitate, which will appear as a fluffy material adhering to the wall of the microcentrifuge tube.

11. Label the tube with your initials or group number.

12. Using a designated pipet, add 1 ml of ice cold 95-100% ethanol or isopropanol. Mix thoroughly by inverting and shaking.

13. Place the tube on ice for 5 minutes.

14. Place the tube (with counterbalance) in the microcentrifuge so the strap which connects the lid to the tube faces the outside of the rotor.

15. Centrifuge at full speed for 10 minutes.

   After centrifugation, a small pellet should be visible in the lower part of the tube.

16. Carefully remove the supernatant with a transfer pipet without touching the inside walls of the tube.

17. Leave the tube open and allow the pellet to air dry for approximately 10 minutes.

18. Resuspend the nucleic acid pellet by adding 50 µl of RNase Solution (Tris-HCl-EDTA buffer containing RNase).

19. Cap the tube and mix by inverting, shaking or vortexing.

20. Briefly centrifuge to get all of the contents to the bottom of the tube.

21. Incubate the tube at 37°C for 10 minutes for the RNase reaction to digest RNAs that would interfere with the gel separation. Plasmid DNA is not affected by RNase since the enzyme is specific for RNA only.

23. Prepare the sample for electrophoresis:
   - Add 5 µl of 10x Gel Loading Solution and mix.
   - Load 40 µl of the prepared sample on a 0.8% UltraSpec-Agarose gel.

**OPTIONAL STOPPING POINT**

Store samples at 4°C until ready for electrophoresis.
Option 2 - Isolation of Plasmid DNA for Restriction Enzyme Digestion

**EXPERIMENT OBJECTIVE:**

In this experiment, you will extract a 3000 base pair plasmid from *E. coli* cells. The plasmid contains a gene for ampicillin resistance and is present in many copies per cell. Using the procedure outlined below, this plasmid can be digested with restriction enzymes.

*Note:* The extracted plasmid does not contain sites for common restriction enzymes suitable for mapping purposes. EDVOTEK recommends Cat. #s 206 or 306 which are experiments specifically designed for mapping.

**LABORATORY SAFETY**

This experiment is designed for staining of DNA with either DNA Blue InstaStain™ or Methylene Blue Plus™ stain after electrophoresis. As with any biological stain, care should be taken when handling solutions or gels containing methylene blue. Gloves and goggles should be worn when handling staining reagents, and worn routinely throughout the experiment as good laboratory practice.

**ISOLATION OF PLASMID DNA FOR RESTRICTION ENZYME DIGESTION WITH ECO RI**

1. Obtain a microcentrifuge tube of suspended *E. coli* cells and put your initials or group number on it. Place the tube on ice.

2. Using a designated 1 ml pipet, add 0.4 ml of Cell Lysis Solution (contains sodium hydroxide and SDS). This step will denature the proteins.

3. Cap the tube and gently mix by inverting the tube 5 times.

4. Keep on ice for 5 minutes.

5. Using a designated 1 ml pipet, add 0.3 ml of potassium acetate neutralization buffer (D). Potassium salt of SDS will precipitate from solution in the cold. This step will remove the SDS.

6. Cap the tube and mix thoroughly by inverting the tube. A white precipitate should form.

7. Keep the tube on ice for 4 minutes.
Isolation of Plasmid DNA for restriction enzyme digestion, continued

8. Place the tube in a microcentrifuge with a counterbalance. (Another group’s tube will serve this purpose, or a tube containing 0.7 ml of water.)

9. Centrifuge at full speed (10,000 to 14,000 rpm) for 5 minutes.

10. Transfer 0.5 ml of the supernatant to a clean microcentrifuge tube. The supernatant contains the plasmid to be further purified.

   Try to avoid transferring any of the precipitate, which will appear as a fluffy material adhering to the wall of the microcentrifuge tube.

11. Mix the Deproteinization Matrix thoroughly and add 0.5 ml to the tube containing 0.5 ml of plasmid DNA solution.

12. Mix vigorously (or vortex) for 3 minutes.

13. Centrifuge at maximum speed for 5 minutes.

14. Transfer 0.5 ml supernatant to a clean microcentrifuge tube and add 1 ml of 95-100% Ethanol or Isopropanol. Mix by inverting and shaking.

15. Place on ice for 5 minutes.

16. Centrifuge at maximum speed for 10 minutes.

17. Carefully remove the supernatant with a transfer pipet without touching the inside walls of the tube.

   Rinse pellet with 1 ml of ice cold 80% ethanol to remove salts. (Be careful - pellet may dislodge.) Remove ethanol with a transfer pipet.

18. Leave the tube open and allow the pellet to air dry for approximately 10 minutes.

19. Resuspend the nucleic acid pellet by adding 50 µl of RNase Solution (Tris-HCl-EDTA buffer containing RNase, 0.08 mg/ml).

20. Cap the tube and mix by inverting, shaking or vortexing.

21. Briefly centrifuge to get all contents to the bottom of the tube.

22. Incubate the tube at 37°C for 20 minutes for the RNase reaction to digest RNAs that would interfere with the gel separation. Plasmid DNA is not affected since the enzyme is specific for RNA only.

23. Freeze sample(s) to store, or proceed with restriction enzyme analysis. Assume plasmid DNA concentration to be approximately 0.02 µg/µl or 1 µg per tube of isolated plasmid DNA.
EXPERIMENTAL PROCEDURES

Agarose Gel Electrophoresis

PREPARING THE GEL BED

Using 7 x 7 cm Gel Beds

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
   
   A. Using Rubber dams:
      • Place a rubber dam on each end of the bed. Make sure the rubber dam sits firmly in contact with the sides and bottom of the bed.
   
   B. Taping with labeling or masking tape:
      • With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
      • Fold the extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.

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

Using the 7 x 15 cm Gel Bed for Two Gels

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

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

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

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

This experiment requires a 0.8% gel.

3. Use a 250 ml flask to prepare the diluted gel buffer.
   • With a 1 ml pipet, measure the buffer concentrate and add the distilled water as indicated in Table A.

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

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

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

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

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

7. Cool the agarose solution to 55°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 5.
Casting Individual 0.8% Gels, continued

Caution!

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

EXPERIMENTAL PROCEDURES

After the gel is cooled to 55°C:

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

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

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

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

PREPARING THE GEL FOR ELECTROPHORESIS

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

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

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

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

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

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

Table B: Electrophoresis (Chamber) Buffer

<table>
<thead>
<tr>
<th>EDVOTEK Model #</th>
<th>Concentrated Buffer (50x) + Distilled Water = Total Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6</td>
<td>4 ml 196 ml 200 ml</td>
</tr>
<tr>
<td>M6 +</td>
<td>6 ml 294 ml 300 ml</td>
</tr>
<tr>
<td>M12, M20</td>
<td>8 ml 392 ml 400 ml</td>
</tr>
<tr>
<td>M36</td>
<td>10 ml 490 ml 500 ml</td>
</tr>
</tbody>
</table>

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

Reminder:

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

Have a waterbath or beaker of water warmed to 65°C for heating the tubes containing DNA fragments before gel loading. At 65°C, non-specific aggregation due to sticky ends generated by restriction enzyme digestions will melt. This will result in sharp individual DNA bands upon separation by agarose gel electrophoresis.

LOADING DNA SAMPLES

1. Each group should load 40 µl of the prepared plasmid DNA sample in a well of the agarose gel.

2. Remember to note the well in which each group loaded its sample.

RUNNING THE GEL

1. After the samples are loaded, carefully snap the cover down onto the electrode terminals.

   Make sure that the negative and positive indicators on the cover and apparatus chamber are properly oriented.

2. Insert the plug of the black wire into the black input of the power source (negative input).
   Insert the plug of the red wire into the red input of the power source (positive input).

3. Set the power source at the required voltage and run the electrophoresis for the length of time as determined by your instructor. General guidelines are presented in Table C at left.

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

5. Allow the tracking dye to migrate 3.5 to 4 centimeters from the wells for adequate separation of the DNA bands.

6. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.

7. Proceed to instructions for staining the gel for DNA Visualization with either DNA Blue InstaStain™ or Methylene Blue Plus™.

---

### Table C: Time and Voltage

<table>
<thead>
<tr>
<th>Volts</th>
<th>Recommended Time</th>
<th>Minimum</th>
<th>Optimal</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>60 min</td>
<td>2.0 hrs</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>40 min</td>
<td>1.5 hrs</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>30 min</td>
<td>45 min</td>
<td></td>
</tr>
</tbody>
</table>

*The EDVOTEK Model #M6 should not be run at higher than 70 volts.*
Staining & Visualization of DNA

DNA BLUE INSTASTAIN™

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

Staining with DNA Blue InstaStain™

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

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

3. Firmly run your fingers over the entire surface of the DNA InstaStain. Do this several times.

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

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

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

Advantages of DNA Blue InstaStain™ vs. Liquid Staining

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

WEAR SAFETY GOGGLES AND GLOVES
Destaining and Visualization of DNA

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

6. Conduct destaining with distilled water that has been warmed to 37°C.
   - First destain: submerge the gel under a small amount of 37°C distilled water for 15 minutes with occasional agitation.
   - Second destain: submerge the gel under a small amount of 37°C distilled water for another 15 minutes with occasional agitation.

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

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

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

Storage and Disposal of Gel

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

  DO NOT FREEZE AGAROSE GELS.

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

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

Useful Hint!

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

WEAR SAFETY GOGGLES AND GLOVES

Remember!

Dilution of Methylene Blue Plus™ stain:

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

EXPERIMENTAL PROCEDURES

TRADITIONAL LIQUID STAINING WITH METHYLENE BLUE PLUS™

1. Remove each gel from its bed and totally submerse the gel(s) in one tray containing 600 ml of diluted Methylene Blue Plus™ stain. Do not stain gel(s) in the electrophoresis apparatus.

2. Stain gel(s) for a minimum of 30 minutes, with occasional agitation.

3. Conduct destaining twice in 600 ml of distilled water that has been warmed to 37°C.
   - First destain: completely submerge the gel(s) in 600 ml of 37°C distilled water for 15 minutes with occasional agitation. Then discard the destaining solution.
   - Second destain: completely submerge the gel(s) in 600 ml of 37°C distilled water for another 15 minutes with occasional agitation.

   Bands will become clearly visible after the second destain. You may also leave the gel(s) in destain overnight.

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

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

Storage and Disposal of Gel

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

  DO NOT FREEZE AGAROSE GELS.

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

1. What are some reasons for isolating plasmid DNA?

2. What are the functions of sodium hydroxide and SDS in the cell lysis solution? What is the function of the potassium acetate solution?

3. What structural property of plasmid DNA allows it to be separated from chromosomal DNA during alkaline cell lysis?

4. Was more than one band observed in your plasmid sample after electrophoresis and staining?
## Material Safety Data Sheet

**Tris-EDTA Buffer (TE)**

### Section I - Identification

**Manufacturer's Name:** EDVOTEK, Inc.

**Address:** 14676 Rothgeb Drive

**City, State, Zip Code:** Rockville, MD 20850

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

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

**Date Prepared:** 8-8-98

**Signature of Preparer:**

### Section II - Hazardous Ingredients/Identity Information

**Chemical Identity; Common Name(s):**

- Carboxymethyl cellulose
- Tris(hydroxymethyl)aminomethane
- Disodium EDTA

**CAS #:** 139-33-3

**Other Limits Recommended:**

- OSHA PEL
- ACGIH TLV

### Section III - Physical/Chemical Characteristics

**Boiling Point:** No data

**Specific Gravity:** No data (H₂O = 1)

**Vapor Pressure:** No data (mm Hg)

**Vapor Density:** No data (AIR = 1)

**Evaporation Rate:** No data (Butyl Acetate = 1)

**Appearance and Odor:** Clear, liquid, slight vinegar odor

**Solubility in Water:** Appreciable (greater than 10%) Soluble

### Section IV - Physical/Chemical Characteristics

**Flammable Limits:**

- Lower Explosive Limit (LEL): No data
- Upper Explosive Limit (UEL): No data

**Extinguishing Media:**

- Use extinguishing media appropriate for surrounding fire.

**Safe Handling and Use:**

- Wear protective equipment and SCBA with full facepiece.
- Avoid eye and skin contact.

**Other Precautions:**

- Observe federal, state, and local laws.
- Treat symptomatically and supportively.

**Stable:** Yes

**Incompatibility:**

- Acids, aluminum, metals, oxidizers (strong)
- Independently of any other substances

**Hazardous Decomposition or Byproducts:**

- Thermal decomposition products of toxic and hazardous oxides of C, N, & Na

**Hazardous Polymerization:**

- May Occur

### Section V - Reactivity Data

**Stability:**

- Unstable

**Conditions to Avoid:**

- Excessive heat, sparks or open flame

**Incompatibility:**

- Acids, aluminum, metals, oxidizers (strong)

**Hazardous Decomposition or Byproducts:**

- Thermal decomposition products of toxic and hazardous oxides of C, N, & Na

**Hazardous Polymerization:**

- May Occur

### Section VI - Health Hazard Data

**Route(s) of Entry:**

- Inhalation: Yes
- Skin: Yes
- Ingestion: Yes

**Health Hazards (Acute and Chronic):**

- None

**Carcinogenicity:**

- None identified

**IARC Monographs:**

- None

**OSHA Regulation:**

- None

**Signs and Symptoms of Exposure:**

- Inflammation of respiratory tract, skin, eyes

**Medical Conditions Generally Aggravated by Exposure:**

- None

**Medical Conditions Generally Aggravated by Exposure:**

- Renal or heart disease, potassium deficiency, insulin dependent, diabetes, seizures or intracranial lesions

**Emergency First Aid Procedures:**

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

**Waste Disposal Method:**

- Dispose in accordance with all applicable federal, state, and local environmental regulations.

**Precautions to be Taken in Handling and Storage:**

- Avoid eye and skin contact.

**Other Precautions:**

- Observe federal, state, and local laws.
- Treat symptomatically and supportively.

**Stable:** Yes

**Incompatibility:**

- Acids, aluminum, metals, oxidizers (strong)

**Hazardous Decomposition or Byproducts:**

- Thermal decomposition products of toxic and hazardous oxides of C, N, & Na

**Hazardous Polymerization:**

- May Occur

### Section VII - Control Measures

**Respiratory Protection (Specify Type):**

- Chemical cartridge respirator with full facepiece and organic vapor cartridge

**Ventilation:**

- Local Exhaust: Yes
- Mechanical (General): None

**Protective Gloves:**

- Yes

**Protective Clothing or Equipment:**

- None

**Work/Hygienic Practices:**

- None

**Stable:** Yes

**Incompatibility:**

- Acids, aluminum, metals, oxidizers (strong)

**Hazardous Decomposition or Byproducts:**

- Thermal decomposition products of toxic and hazardous oxides of C, N, & Na

**Hazardous Polymerization:**

- May Occur

### Section VIII - Control Measures

**Respiratory Protection (Specify Type):**

- Chemical cartridge respirator with full facepiece and organic vapor cartridge

**Ventilation:**

- Local Exhaust: Yes
- Mechanical (General): None

**Protective Gloves:**

- Yes

**Protective Clothing or Equipment:**

- None

**Work/Hygienic Practices:**

- None

**Stable:** Yes

**Incompatibility:**

- Acids, aluminum, metals, oxidizers (strong)

**Hazardous Decomposition or Byproducts:**

- Thermal decomposition products of toxic and hazardous oxides of C, N, & Na

**Hazardous Polymerization:**

- May Occur

### Section IX - Other Information

**Emergency Procedures:**

- Emergency eye wash should be available

**Stable:** Yes

**Incompatibility:**

- Acids, aluminum, metals, oxidizers (strong)

**Hazardous Decomposition or Byproducts:**

- Thermal decomposition products of toxic and hazardous oxides of C, N, & Na

**Hazardous Polymerization:**

- May Occur

### Section X - Disposal Considerations

**Waste Disposal Method:**

- Dispose in accordance with all applicable federal, state, and local environmental regulations.

**Precautions to be Taken in Handling and Storage:**

- Avoid eye and skin contact.

**Other Precautions:**

- Observe federal, state, and local laws.
- Treat symptomatically and supportively.
**Material Safety Data Sheet**

**IDENTITY (As Used on Label and List)**

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

### Section I - Identification

<table>
<thead>
<tr>
<th>Manufacturer's Name</th>
<th>EDVOTEK, Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Address</td>
<td>14676 Rothgeb Drive</td>
</tr>
<tr>
<td></td>
<td>Rockville, MD 20850</td>
</tr>
</tbody>
</table>

### Section II - Hazardous Ingredients/Identify Information

<table>
<thead>
<tr>
<th>Chemical Identity; Common Name(s)</th>
<th>OSHA PEL</th>
<th>ACGIH TLV</th>
<th>Recommended % (Optional)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Acetate</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
</tr>
</tbody>
</table>

### Section III - Physical/Chemical Characteristics

<table>
<thead>
<tr>
<th>Boiling Point</th>
<th>For 1% solution 194°F</th>
<th>Specific Gravity (H2O = 1)</th>
<th>No data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vapor Pressure (mm Hg.)</td>
<td>No data</td>
<td>Melting Point</td>
<td>No data</td>
</tr>
<tr>
<td>Vapor Density (AIR = 1)</td>
<td>No data</td>
<td>Evaporation Rate (Butyl Acetate = 1)</td>
<td>No data</td>
</tr>
<tr>
<td>Solubility in Water</td>
<td>Insoluble - cold</td>
<td>Appearance and Odor</td>
<td>White powder, no odor</td>
</tr>
</tbody>
</table>

### Section IV - Physical/Chemical Characteristics

<table>
<thead>
<tr>
<th>Flash Point (Method Used)</th>
<th>No data</th>
<th>Flammable Limits</th>
<th>LEL</th>
<th>N.D.</th>
<th>UEL</th>
<th>N.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extinguishing Media</td>
<td>Water spray, dry chemical, carbon dioxide, halon or standard foam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Special Fire Fighting Procedures</td>
<td>Possible fire hazard when exposed to heat or flame</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unusual Fire and Explosion Hazards</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Section V - Reactivity Data

<table>
<thead>
<tr>
<th>Stability</th>
<th>Unstable</th>
<th>Conditions to Avoid</th>
<th>Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stable</td>
<td>X</td>
<td>None</td>
</tr>
</tbody>
</table>

### Section VI - Health Hazard Data

<table>
<thead>
<tr>
<th>Route(s) of Entry:</th>
<th>Inhalation?</th>
<th>Yes</th>
<th>Skin?</th>
<th>Yes</th>
<th>Ingestion?</th>
<th>Yes</th>
</tr>
</thead>
</table>

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

**Carcinogenicity:**
- NTP: No data
- IARC Monographs: No data
- OSHA Regulation: No data

### Section VII - Precautions for Safe Handling and Use

**Steps to be Taken if 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

### Section VIII - Control Measures

**Respiratory Protection (Specify Type)**
- Chemical cartridge respirator with full facepiece

**Ventilation**
- Local Exhaust
- Special
- Mechanical (General), dilution ventilation

**Protective Gloves**
- Yes
- Splash proof goggles

**Other Protective Clothing or Equipment**
- Impervious clothing to prevent skin contact

**Work/Hygienic Practices**
- None

---

**Material Safety Data Sheet**

**IDENTITY (As Used on Label and List)**

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

### Section I - Identification

<table>
<thead>
<tr>
<th>Manufacturer's Name</th>
<th>EDVOTEK, Inc.</th>
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<tr>
<td>Address</td>
<td>14676 Rothgeb Drive</td>
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<td>Rockville, MD 20850</td>
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### Section II - Hazardous Ingredients/Identify Information

<table>
<thead>
<tr>
<th>Chemical Identity; Common Name(s)</th>
<th>OSHA PEL</th>
<th>ACGIH TLV</th>
<th>Recommended % (Optional)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Acetate</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
</tr>
</tbody>
</table>

### Section III - Physical/Chemical Characteristics

<table>
<thead>
<tr>
<th>Boiling Point</th>
<th>For 1% solution 194°F</th>
<th>Specific Gravity (H2O = 1)</th>
<th>No data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vapor Pressure (mm Hg.)</td>
<td>No data</td>
<td>Melting Point</td>
<td>No data</td>
</tr>
<tr>
<td>Vapor Density (AIR = 1)</td>
<td>No data</td>
<td>Evaporation Rate (Butyl Acetate = 1)</td>
<td>No data</td>
</tr>
<tr>
<td>Solubility in Water</td>
<td>Insoluble - cold</td>
<td>Appearance and Odor</td>
<td>Clear liquid, vinegar-like odor</td>
</tr>
</tbody>
</table>

### Section IV - Physical/Chemical Characteristics

<table>
<thead>
<tr>
<th>Flash Point (Method Used)</th>
<th>No data</th>
<th>Flammable Limits</th>
<th>LEL</th>
<th>No data</th>
<th>UEL</th>
<th>No data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extinguishing Media</td>
<td>Dry chemical, carbon dioxide, water spray or foam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Special Fire Fighting Procedures</td>
<td>Move container from fire area if possible. Avoid breathing vapors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unusual Fire and Explosion Hazards</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Section V - Reactivity Data

<table>
<thead>
<tr>
<th>Stability</th>
<th>Unstable</th>
<th>Conditions to Avoid</th>
<th>Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stable</td>
<td>X</td>
<td>None</td>
</tr>
</tbody>
</table>

### Section VI - Health Hazard Data

<table>
<thead>
<tr>
<th>Route(s) of Entry:</th>
<th>Inhalation?</th>
<th>Yes</th>
<th>Skin?</th>
<th>Yes</th>
<th>Ingestion?</th>
<th>Yes</th>
</tr>
</thead>
</table>

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

**Carcinogenicity:**
- NTP: No data
- IARC Monographs: No data
- OSHA Regulation: No data

### Section VII - Precautions for Safe Handling and Use

**Steps to be Taken if Material is Released for Spilled**
- Mop up with absorbent material and dispose of properly

**Waste Disposal Method**
- Follow all federal, state, and local regulations

**Precautions to be Taken in Handling and Storing**
- Wear eye protection

### Section VIII - Control Measures

**Respiratory Protection (Specify Type)**
- SCBA with full facepiece

**Ventilation**
- Local Exhaust
- Special
- Mechanical (General), dilution ventilation

**Protective Gloves**
- None
- Splash proof goggles

**Other Protective Clothing or Equipment**
- Not required

**Work/Hygienic Practices**
- Avoid contact
### Material Safety Data Sheet

#### Section I - Identity (As Used on Label and List)

**Manufacturer's Name:** EDVOTEK, Inc.  
**Address:** 14676 Rothgeb Drive, Rockville, MD 20850

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

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

**Date Prepared:** 6/25/97  
**Signature of Preparer (optional):**

---

#### Section II - Hazardous Ingredients/Identify Information

**Hazardous Components (specific Chemical Identity, Common Name(s)):**

<table>
<thead>
<tr>
<th>Chemical Identity</th>
<th>CAS Number</th>
<th>OSHA PEL</th>
<th>ACGIH TLV</th>
<th>Other Limits Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Dodecyl Sulfate (SDS)</td>
<td>151-21-3</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
</tr>
</tbody>
</table>

---

#### Section III - Physical/Chemical Characteristics

**Boiling Point:** No data  
**Vapor Pressure (mm Hg.):** No data  
**Vapor Density (AIR = 1):** No data  
**Solubility in Water:** Soluble  
**Appearance and Odor:** Blue liquid, no odor

---

#### Section IV - Physical/Chemical Characteristics

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

**Extinguishing Media:** Dry chemical, 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:** Unstable  
**Conditions to Avoid:** None

**Incompatibility:** Strong oxidizing agents

**Hazardous Decomposition or Byproducts:** Carbon monoxide, carbon dioxide, sulfide oxides

**Hazardous Polymerization:** May Occur  
**Conditions to Avoid:** None

---

#### Section VI - Health Hazard Data

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

**Health Hazards (Acute and Chronic):** May cause irritation to eyes, ears and nose.

**Carcinogenicity:** NTP? Yes IARC Monographs? Yes OSHA Regulation? Yes

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

**Medical Conditions Generally Aggravated by Exposure:** None

**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:**
- Wear eye and skin protection and mop spill area. Rinse with water.
- Waste Disposal Method:
  - Observe all federal, state, and local regulations.

**Special 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: Local Exhaust  
- Mechanical (General) Yes

**Protective Gloves:** Yes

**Protective Clothing or Equipment:** None required  
**Work/Hygienic Practices:** Avoid eye and skin contact

---

### Material Safety Data Sheet

#### Section I - Identity (As Used on Label and List)

**Manufacturer's Name:** EDVOTEK, Inc.  
**Address:** 14676 Rothgeb Drive, Rockville, MD 20850

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

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

**Date Prepared:** 6/25/97  
**Signature of Preparer (optional):**

---

#### Section II - Hazardous Ingredients/Identify Information

**Hazardous Components (specific Chemical Identity, Common Name(s)):**

<table>
<thead>
<tr>
<th>Chemical Identity</th>
<th>CAS Number</th>
<th>OSHA PEL</th>
<th>ACGIH TLV</th>
<th>Other Limits Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Dodecyl Sulfate, Sodium</td>
<td>151-21-3</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
</tr>
</tbody>
</table>

---

#### Section III - Physical/Chemical Characteristics

**Boiling Point:** No data  
**Vapor Pressure (mm Hg.):** No data  
**Vapor Density (AIR = 1):** No data  
**Solubility in Water:** Soluble  
**Appearance and Odor:** Clear liquid, no odor

---

#### Section IV - Physical/Chemical Characteristics

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

**Extinguishing Media:** Water spray, dry chemical powder

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

**Unusual Fire and Explosion Hazards:** May emit toxic fumes

---

#### Section V - Reactivity Data

**Stability:** Unstable  
**Conditions to Avoid:** None

**Incompatibility:** Strong oxidizing agents

**Hazardous Decomposition or Byproducts:** Sulfur oxides, and bromides

**Hazardous Polymerization:** May Occur  
**Conditions to Avoid:** None

---

#### Section VI - Health Hazard Data

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

**Health Hazards (Acute and Chronic):** May cause irritation to eyes, ears and nose.

**Carcinogenicity:** NTP? Yes IARC Monographs? Yes OSHA Regulation? Yes

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

**Medical Conditions Generally Aggravated by Exposure:** None

**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:**
- Wear eye and skin protection and mop spill area. Rinse with water.
- Waste Disposal Method:
  - Observe all federal, state, and local regulations.

**Special 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: Local Exhaust  
- Mechanical (General) None

**Protective Gloves:** None

**Protective Clothing or Equipment:** None required  
**Work/Hygienic Practices:** Avoid eye and skin contact
Material Safety Data Sheet

IDENTITY (As Used on Label and List) 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 - Identification Information
Manufacturer's Name: EDVOTEK, Inc.
Address: 14676 Rothgeb Drive
City: Rockville
State: MD
Zip Code: 20850

Emergency Telephone Number:
(301) 251-5990

Date Prepared: 12/18/97
Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components: Nuclease, ribo
CAS # 61-73-4

Section III - Physical/Chemical Characteristics

Appearance and Odor
Clear liquid, no odor

Boiling Point
50°C

Vapor Pressure (mm Hg.)
No data

Vapor Density (AR = 1)
No data

Solubility in Water
Soluble

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)
No data

Solubility in Water
Soluble - cold

Section V - Reactivity Data

Stability
Unstable

Conditions to Avoid
None

Incompatibility
None

Hazardous Decomposition or Byproducts
None

Polymization
May Occur

Condition to Avoid
None

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic)
None

Carcinogenicity:
NTP: No data
OSHA Regulation: Yes

Signs and Symptoms of Exposure
Unknown

Medical Conditions Generally Aggravated by Exposure
Unknown

Emergency First Aid Procedures
Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Wear SCBA and protective clothing to prevent contact with skin and eyes.

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

Section VIII - Control Measures

Respiratory Protection (Specify Type)
NIOSH-MSHA approved respirator

Ventilation
None

Protective Gloves
Chemical resistant

Protective Clothing or Equipment
Yes

Work/Hygienic Practices
Avoid contact and inhalation

Material Safety Data Sheet

IDENTITY (As Used on Label and List) 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 - Identification Information
Manufacturer's Name: DNA Blue InstaStainTM
Address: 14676 Rothgeb Drive
City: Rockville
State: MD
Zip Code: 20850

Emergency Telephone Number:
(301) 251-5990

Date Prepared: 01/31/00
Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components: Methylene blue
CAS # 68-61-7

Section III - Physical/Chemical Characteristics

Boiling Point
No data

Vapor Pressure (mm Hg.)
No data

Vapor Density (AR = 1)
No data

Solubility in Water
Soluble - cold

Section IV - Physical/Chemical Characteristics

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

Special Fire Fighting Procedures
Self contained breathing apparatus and protective clothing to prevent contact with skin and eyes

Unusual Fire and Explosion Hazards
Emits toxic fumes under fire conditions

Section V - Reactivity Data

Stability
Unstable

Conditions to Avoid
None

Incompatibility
Strong oxidizing agents

Hazardous Decomposition or Byproducts
None

Polymization
May Occur

Condition to Avoid
None

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic)
None

Carcinogenicity:
NTP: No data
OSHA Regulation: Yes

Signs and Symptoms of Exposure
None

Medical Conditions Generally Aggravated by Exposure
None

Emergency First Aid Procedures
Treat symptomatically

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled
Mop up with absorbent material. Dispose of properly.

Waste Disposal Method
Follow all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing
Avoid eye and inhalation.

Other Precautions
None

Section VIII - Control Measures

Respiratory Protection (Specify Type)
MIOSH/OSHA approved, SCBA

Ventilation
Local Exhaust
None
Special

Protective Gloves
Chemical resistant

Protective Clothing or Equipment
Yes

Work/Hygienic Practices
Yes
Material Safety Data Sheet

IDENTITY (As Used on Label and List)
Sodium Hydroxide

Section I

Manufacturer's Name
EDVOTEK, Inc.
Address
14676 Rothgeb Drive
Rockville, MD 20850

Emergency Telephone Number
(301) 251-5990

Date Prepared
11/4/98

Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Emergency Telephone Number
(301) 251-5990

Telephone Number for information

Date Prepared
11/4/98

Signature of Preparer (optional)

Section III - Physical/Chemical Characteristics

Boiling Point
138°C

Specific Gravity (H₂O = 1)
2.13

Vapor Pressure (mm Hg.)
20°C

Melting Point
318°C

Vapor Density (AIR = 1)
NO data

Evaporation Rate
(Butyl Acetate = 1)
NO data

Solubility in Water
10% appreciable

Appearance and Odor
White pellets, odorless

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)
NA

Flammable Limits
LEL NA

UEL NA

Section V - Reactivity Data

Stability
Unstable

Conditions to Avoid
moisture

Incompatibility
Water, strong acids, metals, combustible materials, organic materials

Zinc, aluminum, peroxide, hydrogen peroxide...

Hazardous Decomposition or Byproducts
None identified

Hazardous Polymerization
May Occur

Conditions to Avoid

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic)

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

Signs and Symptoms of Exposure

Inhalation: irritation
Skin/eye contact: severe irritation or burns

Medical Conditions Generally Aggravated by Exposure
None identified

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled

Waste Disposal Method

Steps to be Taken in Handling and Storing

Other Precautions

Section VIII - Control Measures

Respiratory Protection (Specify Type)
NIOSH/MSHA approved respirator

Ventilation
Local Exhaust Yes

Mechanical (General) Yes

Protective Gloves
Neoprene gloves

Eye Protection Safety goggles

Other Protective Clothing or Equipment Uniform, apron

Work/Hygiene Practices
Avoid contact

The content includes sections on manufacturer information, hazardous ingredients, physical and chemical characteristics, reactivity data, health hazards, precautions, and control measures for the material Sodium Hydroxide. It provides detailed information on handling, storage, and emergency procedures.