The Biotechnology Education Company®

EDVOTEK®

Revised and Updated

EDVO-Kit #

221

Transformation of *E. coli* with pGAL™ (blue colony)

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of bacterial transformation by plasmid DNA. This experiment introduces an opportunity to observe an acquired phenotypic trait of the transformed bacterial cells. The presence of blue bacterial colonies visually demonstrates the expression of a specific gene for the Lac^+ phenotype.

No IPTG used in this experiment.

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.

Table of Contents

Important READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

Experiment Components

Component Quantities:

Experiment # 221 is designed for 10 groups (2-4 students per group).

Contents

Storage: Store components A-G in the refrigerator.

A Transformation LyphoCells™ (DO NOT FREEZE)
B Supercoiled pGAL™ (blue colony)
C Control Buffer (no DNA)
D Ampicillin
E X-Gal in solvent (pre-measured)**
F Cell reconstitution medium
G Solvent for induction of competency

Storage: Store components listed below at Room temperature

- Bottle ReadyPour™ Luria Broth Agar, sterile (also referred to as ReadyPour medium)
- Bottle Luria Broth Medium for Recovery, sterile (also referred to as Luria Recovery Broth)
- Petri plates
- Plastic microtipped transfer pipets
- inoculating loops (sterile)
- Microtest tubes with attached lids

**EDVOTEK's solvent formulation offers a safer alternative to the usual solvent, N, N-dimethylformamide (DMF).

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.

None of the experiment components are derived from human sources.

Requirements

- Automatic Micropipet (5-50 µl) and tips
- Two Water baths (37°C and 42°C)
- Thermometer
- Incubation Oven (34°C and 37°C)
- Pipet pumps or bulbs
- Ice
- Marking pens
- Bunsen burner, hot plate or microwave oven
- Hot gloves

* If a second water bath is not available, water can be heated to 42°C in a beaker. The cells will require this temperature for only a few minutes. Alternatively, 42°C water can be put in a small styrofoam container with a cover. The temperature needs to be held at 42°C.

Technical Service Department

EDVO-TECH SERVICE
1-800-EDVOTEK
(1-800-338-6835)
Mon - Fri 9 am - 6 pm ET

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:

- The experiment number and title
- Kit Lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date

Bacterial Transformation

Bacterial transformation is of central importance in molecular biology. It allows for the introduction of genetically engineered or naturally occurring plasmids in bacterial cells. This makes possible the propagation, genetic expression and isolation of DNA plasmids.

The transformation process involves the uptake of exogenous DNA by cells which results in a newly acquired genetic trait that is stable and heritable. Bacterial cells must be in a particular physiological state before they can be transformed. This state is referred to as competency. Competency can occur naturally in certain species of *Haemophilus* and *Bacillus* when the levels of nutrients and oxygen are low. Competent *Haemophilus* expresses a membrane associated transport complex which binds and transfers certain DNA molecules from the medium into the cell where they are incorporated and their genes are expressed. In nature, the source of external DNA is from other cells.

Most of the current transformation experiments involve *E. coli*. This organism does not enter a stage of competency unless artificially induced. Treatment to achieve competency involves the use of chloride salts, such as calcium chloride, and sudden hot and cold temperature changes. The metal ions and temperature changes affect the structure and permeability of the cell wall and membrane so that DNA molecules can be absorbed by the bacteria. The mechanism of DNA transport in the cell still is not fully understood. Competent *E. coli* cells are fragile and must be treated carefully.

The transformation efficiency is defined by the number of transformants obtained per microgram of DNA. For example, 10 nanograms of DNA were used for a transformation and the cells were allowed to recover in a final volume of 1 ml. One tenth of this volume was plated and produced 100 colonies on a selective agar medium. Therefore, 1000 transformants are present per ml. Keeping in mind that each colony grew from one transformed cell, the efficiency would be 1000/0.01ug = 1 x 10^5. Transformation efficiencies of 10^5 to 10^6 are more than sufficient for most subcloning experiments. When the cloning of single copy genes from genomic DNA is done, the required efficiencies are 10^7 to 10^8.

The determination for transformation efficiency in this case is outlined in Figure 1. Transformation efficiencies gen-

$$\frac{\text{Number of transformants}}{\mu\text{g of DNA}} \times \frac{\text{final vol at recovery (ml)}}{\text{vol plated (ml)}} = \text{Number of transformants per } \mu\text{g}$$

Specific example:

$$\frac{100 \text{ transformants}}{0.01\ \mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml}} = 100{,}000\ (1 \times 10^5) \text{ transformants per } \mu\text{g}$$

Figure 1:
Bacterial Transformation Efficiency Calculation

Bacterial Transformation

Background Information

erally range from 1 x 10^4 to 1 x 10^7 cells per microgram of DNA. There are special procedures which can produce cells having transformation efficiencies approaching 10^{10}. However, transformation is never 100% efficient. Approximately 1 in every 10,000 cells successfully incorporates plasmid DNA in preparations having average competency. However, there is such a large number of cells in a sample (typically 1 x 10^9) that only a small fraction needs to be transformed to obtain colonies on a plate. The same volume of recovered cells plated on selective (contains antibiotic) and nonselective agar medium will yield vastly different numbers of cells. The nonselective medium will have many more growing cells that form a bcterial lawn.

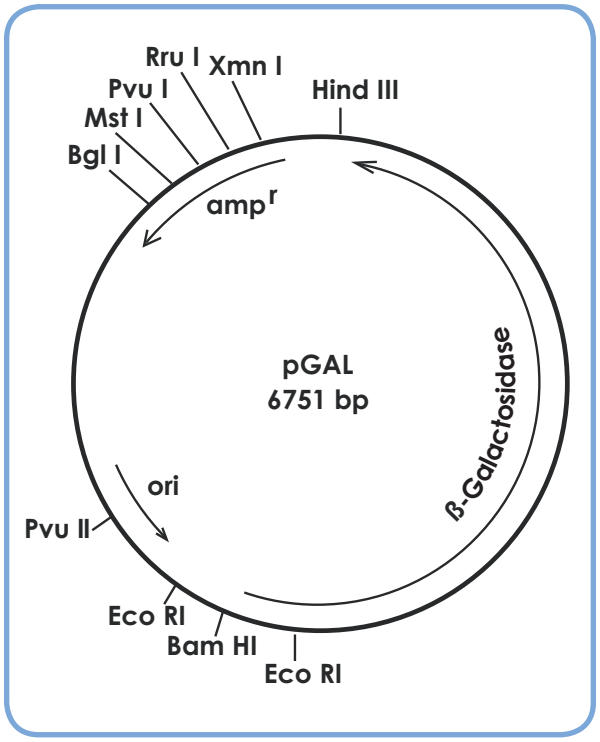


Figure 2 :
DNA map of pGAL
Not all restriction enzymes are shown.

Many different plasmids serve as useful tools in molecular biology. One example is the pGAL plasmid, present in multiple copies in specified host *E. coli* host cells. It contains 6751 base pairs and has been cleverly modified by genetic engineering. In the cell, it **does not** integrate into the bacterial chromosome, but replicates autonomously. The pGAL plasmid contains the *E. coli* gene which codes for β-galactosidase. In the presence of artificial galactosides such as 5-Bromo-4 Chloro 3-indolyl-β-D-galactoside (X-Gal), pGAL colonies appear blue when X-Gal is cleaved by β-galactosidase and forms a colored product.

This experiment has been designed to utilize EDVOTEK Transformation LyphoCells™. It also contains the proprietary plasmid, pGAL (Blue Colony), which was engineered by EDVOTEK. Plasmid pGAL carries the complete gene for β-galactosidase. Since the host *E. coli* does not contain a β-galactosidase gene, only cells transformed by the pGAL plasmid will produce the functional β-galactosidase enzyme. Cells that express β-galactosidase will cleave X-Gal and the pGAL transformed colonies will be blue.

Bacterial Transformation

In addition to the expression and cleavage of X-Gal by β-galactosidase, transformation by pGAL is also demonstrated by resistance to ampicillin. *E. coli* host cells used in this experiment are **not** naturally resistant to ampicillin. The plasmid pGAL contains the gene which encodes for β-lactamase that inactivates ampicillin. *E. coli* cells transformed by pGAL will express the resistance gene product β-lactamase as an extracellular enzyme excreted from *E. coli* cells. Once outside the cell, the enzyme diffuses into the surrounding medium and inactivates ampicillin.

With time, small "satellite" colonies may appear around a large blue colony. Cells in the small "satellite" or "feeder" colonies are not resistant to ampicillin and have not been transformed with the pGAL plasmid. They are simply growing in a region of agar where β-lactamase has diffused and inactivated the antibiotic ampicillin. The number of satellite colonies increases if the concentration of ampicillin is low or the plates have incubated for longer times.

Experiment Overview

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT OBJECTIVE:

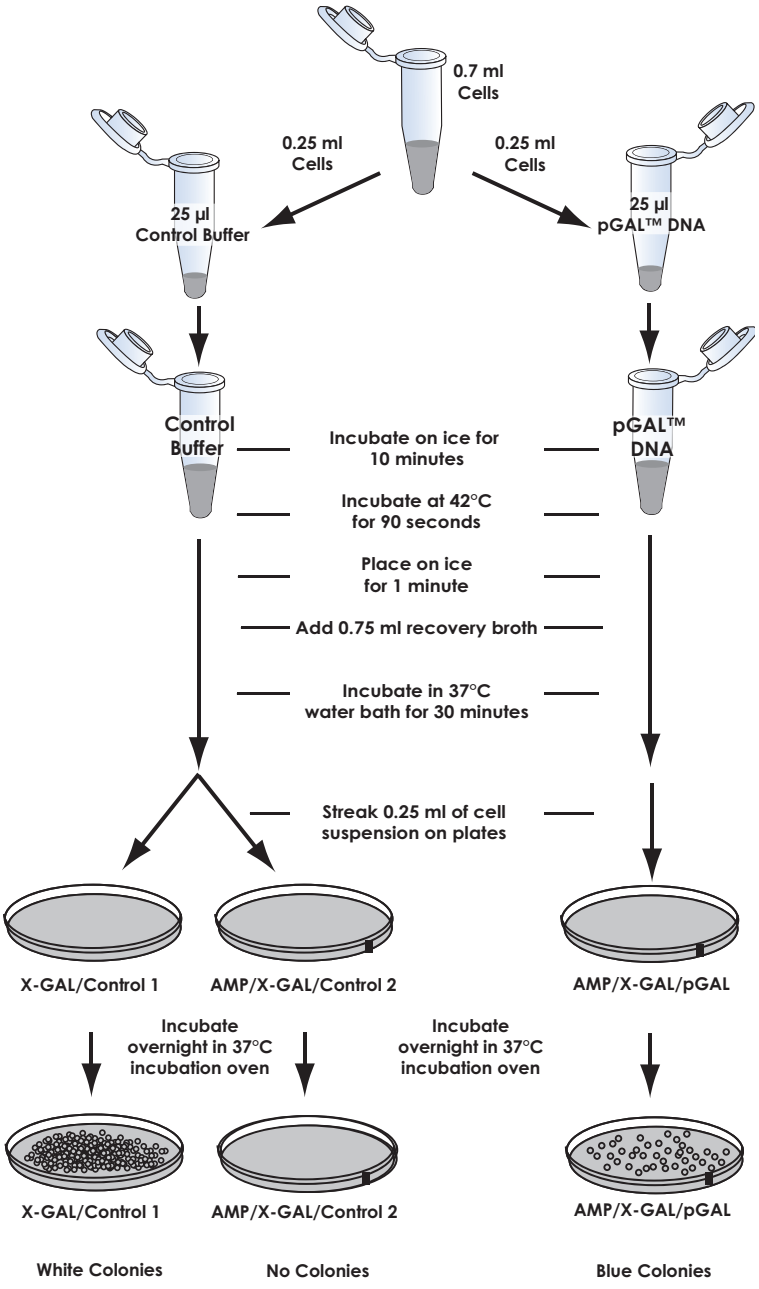
The objective of this experiment module is to develop an understanding of the biologic process of bacterial transformation by plasmid DNA. This experiment demonstrates the acquired Lac^+ phenotypic trait of the transformed bacterial cells as shown by the presence of blue bacterial colonies.

BRIEF DESCRIPTION OF EXPERIMENT:

In this experiment, students will transform host bacterial cells with a plasmid DNA. The transformants acquire antibiotic resistance and exhibit a blue color due to the incorporation and expression of β-galactosidase and ampicillin resistance genes. IPTG is not required since pGAL™ contains the intact β-galactosidase gene. The number of transformants will be counted and the transformation efficiency will be determined.

Experiment Overview

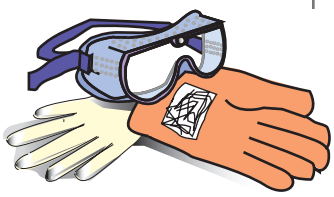
TRANSFORMATION EXPERIMENT FLOW CHART



For optimal results, store covered plates in the upright position after streaking to allow the cell suspension to be absorbed by the agar. After approximately 20 minutes, invert the plates for overnight incubation at 37°C.

Laboratory Safety

The Experiment



Important READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

1. Gloves and goggles should be worn routinely as good laboratory practice.

2. Exercise extreme caution when working with equipment which is used in conjunction with the heating and/or melting of reagents.

3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.

4. The *E. coli* bacteria used in this experiment is not considered pathogenic. Although it is rarely associated with any illness in healthy individuals, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.

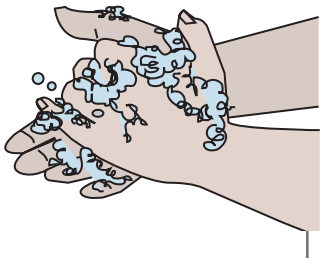
5. Properly dispose materials after completing the experiment:

   A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.

   B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:

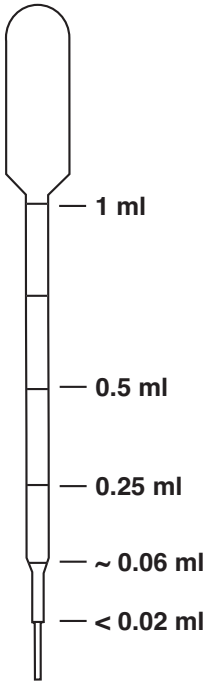
   - Autoclave at 121° C for 20 minutes.
     Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.

   - Soak in 10% bleach solution.
     Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.



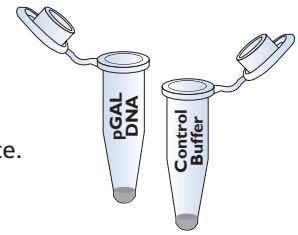
6. Wear gloves, and at the end of the experiment, wash hands thoroughly with soap and water.

Transformation of *E.coli*



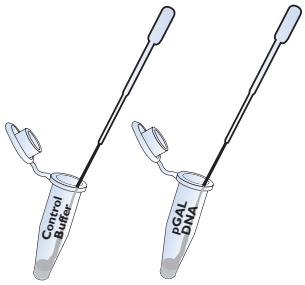
Guide for Sterile Calibrated Transfer Pipet

SETTING UP THE TRANSFORMATION AND CONTROL EXPERIMENT

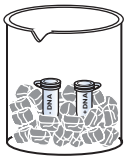


1. Put your initials or group number on the tubes labeled "pGAL DNA" (contains 25 µl of plasmid DNA) and "Control Buffer" (contains 25 µl of buffer) . Place them back on ice.

2. Set up the Control:
   - Using a sterile transfer pipet, transfer 0.25 ml (250 µl) of cell suspension from the tube "Cells" to the tube "Control Buffer".
   - Carefully place the pipet back into the wrapper.
   - Cap the tube; mix by tapping. Put the tube back on ice.



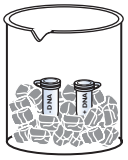
3. Set up the transformation:
   - Using the same pipet from Step 2, transfer 0.25 ml (250 µl) of cell suspension from the tube "Cells" to the tube "pGAL DNA".
   - Cap the tube; mix by tapping. Put the tube back on ice.



4. Incubate the cells prepared in steps 1 - 3 on ice for **10 minutes**.

5. Place both transformation tubes at **42°C for 90 seconds**.

   This heat shock step facilitates the entry of DNA in bacterial cells.



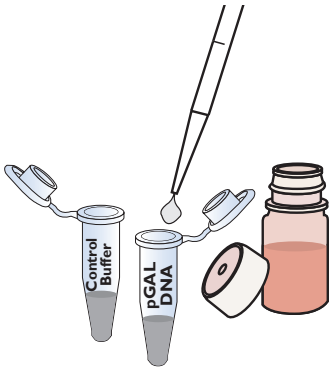
6. Return both tubes to the ice bucket and incubate for for **1 minute**.

Transformation of *E.coli*

7. Add **0.75 ml** of the Recovery Broth to the tube "Control Buffer".

   Add the recovery broth with a sterile 1 ml pipet. **Avoid touching the cells with the pipet.**

8. Add **0.75 ml** of the Recovery Broth to the tube "pGAL DNA".



Quick Reference:

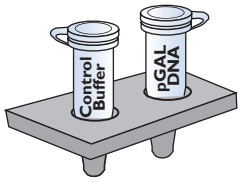
DNA and competent cells are combined in a 0.25 ml suspension. After the cells have incubated with the DNA, growth medium (recovery broth) is added. Bacterial cells continue to grow through the recovery process, during which time the cell wall is repaired. Cells recover and begin to express the antibiotic resistance gene.

9. Incubate the closed tubes in a 37°C water bath for **30 minutes** for a recovery period.

10. While the tubes are incubating, label 3 agar plates as indicated below. Write on the bottom or side of the petri plate.

    - Label one unstriped plate: X-GAL/Control 1
    - Label one striped plate: AMP/X-GAL/Control 2
    - Label one striped plate: AMP/X-GAL/pGAL
    - Put your initials or group number on all the plates.

11. After the recovery period, remove the tubes from the water bath and place them on the lab bench. Proceed to plating the cells for



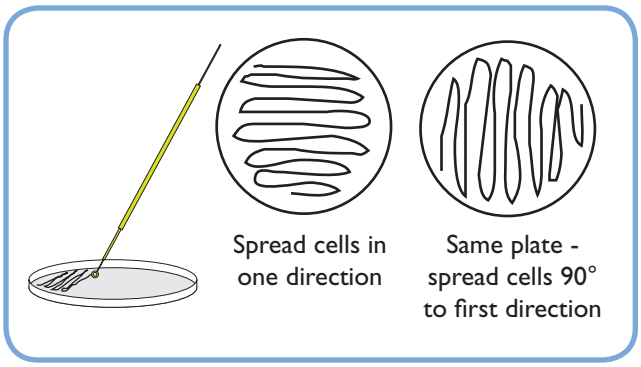
Transformation of *E.coli*

PLATING THE CELLS

Plating cells from the tube labeled "Control":

12. Use a fresh, sterile 1 ml pipet to transfer recovered cells from the tube "Control Buffer" to the middle of the following plates:

    - 0.25 ml to the plate labeled X-GAL/Control 1
    - 0.25 ml to the plate labeled AMP/XGAL/Control 2



13. Spread the cells over the entire plate with a sterile inoculating loop.

14. Cover both control plates and allow the liquid to be absorbed.

To avoid contamination when plating, do not set the lid down on the lab bench -- Lift the lid of the plate only enough to allow spreading. Be careful to avoid gouging the loop into the agar.

Plating cells from the tube labeled "pGAL DNA"

15. Use a fresh, sterile 1 ml pipet to transfer recovered cells from the tube "pGAL DNA" to the middle of the following plate:

    - 0.25 ml to the plate labeled AMP/X-GAL/pGAL

16. Spread the cells with a sterile inoculating loop.

17. Cover the plate and allow the liquid to be absorbed (approximately 15-20 minutes).

Reminder: Follow proper procedures for disposal of contaminated materials.

Transformation of *E.coli*

The Experiment

PREPARING PLATES FOR INCUBATION

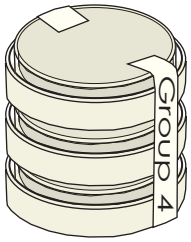
20. Stack your group's set of plates on top of one another and tape them together. Put your initials or group number on the taped set of plates.

    The plates should be left in the upright position to allow the cell suspension to be absorbed by the agar.

21. Place the set of plates in a safe place designated by your instructor.

22. After the cell suspension is absorbed by the agar (approximately 15 -20 minutes), you or your instructor will place the plates in the **inverted** position (agar side on top) in a 37°C bacterial incubation oven for overnight incubation (15-20 hours).

    If the cells have not been absorbed into the medium, it is best to incubate the plates upright. The plates are inverted to prevent condensation on the lid, which could drip onto the culture and may interfere with experimental results.



Reminder: Follow proper procedures for disposal of contaminated materials.

VIEWING PLATES AFTER INCUBATION

23. Proceed to analyzing your results.

24. After analyzing your results, follow proper procedures for disposal of contaminated materials.

Experiment Results and Analysis

LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

- Record (draw) your observations, or photograph the results.

Following the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

ANSWER THESE QUESTIONS BEFORE ANALYZING YOUR RESULTS.

1. On which plate(s) would you find only genetically transformed bacterial cells? Explain.

2. What is the purpose of the control plates? Explain the difference between each and why it is necessary to run each.

3. Why would one compare plates AMP/X-GAL and AMP/X-GAL/pGAL?

Continued

Experiment Results and Analysis

Data Collection

5. Observe the results you obtained on your transformation and control plates.

   Transformation Plate: + DNA

   - AMP/X-GAL/pGAL

   Control Plates: - DNA

   - X-GAL/ Control 1
   - AMP/X-GAL/ Control 2

6. Draw and describe what you observe. For each of the plates, record the following:

   - How much bacterial growth do you observe? Determine a count.
   - What color are the bacteria?
   - Why do different members of your class have different transformation efficiency values?
   - If you did not get any results, what factors could be attributed to this fact?

Experiment Results and Analysis

DETERMINATION OF TRANSFORMATION EFFICIENCY

Transformation efficiency is a quantitative determination of how many cells were transformed per 1 µg of plasmid DNA. In essence, it is an indicator of how well the transformation experiment worked.

You will calculate the transformation efficiency from the data you collect from your experiment.

1. Count the number of colonies on the plate with ampicillin that is labeled:

   AMP/X-GAL/pGAL

   A convenient method to keep track of counted colonies is to mark the colony with a lab marking pen on the outside of the plate.

2. Determine the transformation efficiency using the formula:

$$\frac{\text{Number of transformants}}{\mu\text{g of DNA}} \times \frac{\text{final vol at recovery (ml)}}{\text{vol plated (ml)}} = \text{Number of transformants per } \mu\text{g}$$

Example:

Assume you observed 40 colonies:

$$\frac{40 \text{ transformants}}{0.025\ \mu\text{g}} \times \frac{1.0\ \text{ml}}{0.25\ \text{ml}} = 6400\ (6.4 \times 10^3) \text{ transformants per } \mu\text{g}$$

Quick Reference for Expt. 221

25 ng (0.025 µg) of DNA is used.

The final volume at recovery is 1.0 ml.
The volume plated is 0.25 ml.

Study Questions

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

1. Did you observe any satellite colonies? Why are the satellite, feeder colonies white?
2. Why did the competent cells which did not receive DNA (control) fail to grow on the plates containing ampicillin?
3. Why are there so many cells growing on the X-GAL plate? What color are they?
4. What evidence do you have that transformation was successful?
5. What are some reasons why transformation may be unsuccessful?

Instructor's Guide

Notes to the Instructor:

IMPORTANT READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

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.

The guidelines that are presented in this manual are based on ten laboratory groups consisting of two, or up to four students. The following are implementation guidelines, which 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 available at the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call 1-800-EDVOTEK for help from our knowledgeable technical staff.

Online Ordering now available

www.edvotek.com

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

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:

- The experiment number and title
- Kit Lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date

Day 1: (Prior to the Lab)

- Prepare agar plates
- Prepare LyphoCells™ (overnight incubation).
- Dispense the DNA and control buffer

Day 2: (Day of Lab Experiment)

- Equilibrate water baths at 37°C and 42°C; incubation oven at 37°C
- Students transform cells and plate for overnight incubation.

Day 3: (Day after Lab Experiment)

- Students observe transformants and controls
- Students calculate transformation efficiency
- Follow clean up and disposal procedures as outlined in the Laboratory Safety section.

Instructor's Guide

Notes to the Instructor:

NATIONAL CONTENT AND SKILL STANDARDS

By performing this experiment, students will develop skills necessary to do scientific inquiry, learn new techniques using several types of biotechnology equipment, and will learn standard procedures used in transformation. Analysis of the experiments will provide students the means to transform an abstract concept into a concrete explanation.

APPROXIMATE TIME REQUIREMENTS

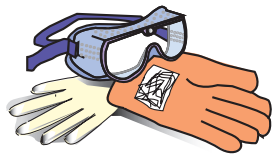
1. The experiment requires the reconstitution and incubation of LyphoCells at 34-37°C for 16-24 hours before the laboratory (overnight incubation). Plan accordingly. For optimal results, incubate LyphoCells at 34-37°C for 19 hours.
2. The agar plates can be prepared several days in advance and stored inverted (agar side on top) in the refrigerator. Preparation requires approximately 1 hour.
3. Dispensing the DNA and control buffer requires approximately 30 minutes. This can be done the day before the lab and stored in the refrigerator.
4. Competent cells must be dispensed just prior to the lab experiment. If tubes are already labeled, dispensing will require approximately 15 minutes.
5. Allow ample time for the equilibration of the water baths at 37°C and 42°C and a bacterial incubation oven at 37°C on the day of the experiment.
6. Each group will perform the transformation experiment and plate a set of three bacterial cells. These procedures require approximately 50 minutes.
7. Overnight incubation of plates is approximately 15-20 hours at 37°C Colonies will also appear between 24 - 48 hours at room temperature.
8. Follow disposal procedures as outlined in the section regarding Laboratory Safety.

LABORATORY NOTEBOOKS

It is highly recommended that students maintain a laboratory notebook to formulate hypotheses and to record experimental procedures and results.

- EDVOTEK Cat. # 1401, Laboratory DataBook is recommended.
- Guidelines for keeping a laboratory notebook is available at the EDVOTEK web site.

Pre-Lab Preparations



Wear Hot Gloves and Goggles during all steps involving heating.

POUR AGAR PLATES
(Prior to the Lab experiment)

- For optimal results, prepare plates two days prior to plating and set aside the plates inverted at room temperature.
- If they are poured more than two days before use, they should be stored inverted in the refrigerator. Remove the plates from the refrigerator and store inverted for two days at room temperature before use.

Heat the ReadyPour™ Medium

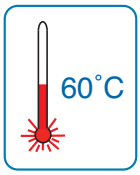
1. Equilibrate a water bath at 60°C for step 5 below.
2. Loosen, but do not remove, the cap on the ReadyPour medium bottle to allow for the venting of steam during heating.

   Caution: Failure to loosen the cap prior to heating or microwaving may cause the ReadyPour medium bottle to break or explode.
3. Squeeze and vigorously shake the plastic bottle to break up the solid agar into chunks
4. Heat the bottle of ReadyPour medium by one of the methods outlined below. When completely melted, the amber-colored solution should appear free of small particles.

   A. Microwave method:
   - Heat the bottle on High for two 30 second intervals.
   - Using a hot glove, swirl and heat on High for an additional 25 seconds, or until all the medium is dissolved.
   - Using a hot glove, occasionally swirl to expedite melting.

   B. Hot plate or burner method:
   - Place the bottle in a beaker partially filled with water.
   - Heat the beaker to boiling over a hot plate or burner.
   - Using a hot glove, occasionally swirl to expedite melting.
5. Allow the melted ReadyPour medium to cool. Placing the bottle in a 60°C water bath will allow the agar to cool, while preventing it from prematurely solidifying.

   When the ReadyPour™ medium reaches approximately 60°C, the bottle will be warm to the touch but not burning hot.



Pre-Lab Preparations

Label ("Stripe") the Plates

6. Use a lab marker to "stripe" the sides of twenty (20) 60 x15 mm petri dishes. This will provide an easy method of differentiating between plates with ampicillin and plates without ampicillin.
   - Open one sleeve of 20 plates and stack the plates neatly.
   - Start the marker at the bottom of the stack and move the marker vertically to the top plate to "stripe" the sides of the 20 plates.
   - These plates will be used for medium with ampicillin.
   - Do not stripe the second sleeve of plates. These will be the control plates.

Pour the Plates (after the medium has cooled)

7 . Thaw and add all of the X-Gal solution (Component E) to the molten and cooled ReadyPour medium. Recap bottle and swirl to mix.

8. Use a fresh 10 ml pipet and pipet pump to pour 10 unstriped plates, 5 ml each. (See Quick Reference: Pouring Agar Plates.) Save the pipet for step 11.

Add reagents to medium which has been cooled. Hot medium will cause reagents such as ampicillin to rapidly decompose.

Quick Reference: Pouring Agar Plates

- Use a sterile 10 ml pipet with a pipet pump to transfer the designated volume of medium to each petri plate. Pipet carefully to avoid forming bubbles.
- Rock the petri plate back and forth to obtain full coverage.
- If the molten medium contains bubbles, they can be removed by passing a flame across the surface of the medium.
- Cover the petri plate and allow the medium to solidify.

Pre-Lab Preparations

9. Add the ampicillin powder (entire contents of tube D) to the remaining molten ReadyPour medium.
10. Recap the bottle; swirl to completely dissolve the ampicillin powder.
11. Use the pipet from step 8 to pour 20 striped plates, 5 ml each. Pour extra plates with any remaining medium.
12. Allow the agar to cool and resolidify.

Note: If plates will be used within two days, store in a sealable plastic bag so the plates will not dry out. Store at room temperature, inverted.

Reminder: Follow proper procedures for disposal of contaminated materials.

Summary of Poured Plates:

10 Agar plates with X-GAL
no stripe plates: 5 ml each - ReadyPour medium without ampicillin

20 Agar plates with X-GAL and AMP
striped plates: 5 ml each - ReadyPour medium with ampicillin

Pre-Lab Preparations

PREPARATION OF COMPETENT CELLS

Day before the Lab

1. Use a 10 ml sterile pipet to add 6 ml sterile cell reconstitution medium (Component F) to the vial of LyphoCells.
2. Replace the rubber stopper and cap. Mix by inverting until the freeze dried plug is dissolved.
3. Shake the cell suspension vigorously and incubate the vial at 34-37°C for 16 - 24 hours (overnight) in an incubation oven. For optimal results, incubate LyphoCells for 19 hours.

Day of the Lab

4. Completely thaw the competency induction solvent (G) and place on ice. (If there is a white precipitate in the bottle, warm it in a 37°C waterbath to dissolve the precipitate.)
5. Mix and resuspend the vial of incubated cells by inverting and gently shaking. Place the vial on ice for 10 minutes.
6. Use a 10 ml sterile pipet to add 3 ml of ice cold competency induction solvent (G) to the vial of cells.

   The competency induction solvent is very viscous. Make sure that a portion of the solvent is not left on the walls of the pipet.
7. Mix the cells and induction solvent thoroughly by inverting the vial several times. The solution should have no dense layers, "streams" or globules (i.e. it should be a uniform suspension).
8. Keep the cells on ice for a minimum of 30 minutes.

   Cells can be kept on ice for up to 3 hours.

Pre-Lab Preparations

Keep cells on ice during dispensing procedures.

Dispensing the Cells just prior to the Experiment

11. Mix the cells by inversion to obtain an even suspension.
12. Use a sterile 1 ml pipet to aliquot 0.7 ml of cells to 10 ice cold tubes labeled "Cells"
13. Cap the tubes and keep them on ice.

PREPARATION OF DNA AND CONTROL BUFFER

1. Place the tubes of supercoiled pGAL DNA (Component B) and control buffer (Component C) on ice.
2. Before dispensing DNA and Control buffer, tap the tubes until all the sample is at the tapered bottom of the tube.

Summary of Reagent Preparations

		Dispense	
10 plates	Agar plates with X-GAL (No Stripe)	5 ml	
20 plates	Agar plates with AMP and X-GAL (Striped)	5 ml	
10 tubes	pGAL DNA	25 µl	on ice
10 tubes	Control Buffer	25 µl	on ice
10 tubes	Cells	0.7 ml	on ice

3. Using an automatic micropipet, dispense 25 µl of the supercoiled pGAL DNA to each of the 10 microtest tubes labeled "pGAL DNA".
4. Cap the tubes and place them on ice.
5. Using a FRESH micropipet tip, dispense 25 µl of control buffer to each of 10 microtest tubes labeled "Control Buffer".
6. Cap the tubes and place them on ice.

Each Group Requires:

1	tube	pGAL™ DNA	25 µl	on ice
1	tube	Cells	0.7 ml	on ice
1	tube	Control Buffer	25 µl	on ice
1	agar plate - no stripe			
2	agar plates - striped			
4	sterile 1 ml pipets			
2	sterile inoculating loops			
1	tube	Recovery broth (optional)	1.5 ml	
2	micropipet tips (if using automatic micropipets)			

Recovery Broth (Optional)

Set up a classroom pipeting station, or dispense recovery broth (optional).

7. Dispense 1.5 ml Recovery Broth into 10 sterile tubes labeled "Recovery Broth" using a sterile pipet.
8. Cap the tubes and place them in the refrigerator if not to be used immediately.

Avoiding Common Pitfalls

1. When heating the ReadyPour medium, make sure it does not boil over and cause the volume to be reduced. Watch the bottle very carefully and remove it from heat if you see signs of the medium boiling over.
2. If the plates are made fresh, the plated cells will take longer to be absorbed into the medium. Invert the plates only after the cell suspension has been absorbed.
3. Do not discard the tubes containing transformed bacteria. After plating an aliquot on selection plates, set the tubes in a rack and leave on the lab bench overnight. If for some reason, transformants do not grow on the selection plates, the cell pellet can be plated as outlined below.

- Collect the bacterial cell pellet by centrifugation in a microcentrifuge. If a microcentrifuge is not available, let the bacteria collect by gravity and do not disturb.
- Remove all except 0.1 to 0.2 ml of medium (supernatant).
- Resuspend cell pellet in remaining medium.
- Spread entire contents of tube on selection medium.
- Incubate plate as before, 15 to 24 hours at 37°C.

Technical Service Department

EDVO-TECH SERVICE
1-800-EDVOTEK
(1-800-338-6835)
Mon - Fri 9 am - 6 pm ET

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:

- The experiment number and title
- Kit Lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date

Experiment Results and Analysis

ANSWER THESE QUESTIONS BEFORE ANALYZING YOUR RESULTS.

1. **On which plate(s) would you find only genetically transformed bacterial cells? Explain.**

   The bacteria growing on the plate labeled AMP/X-GAL/pGAL are transformed cells since only those cells that have taken up the plasmid which expresses the ampicillin resistance gene and the fluorescent gene(s) will survive on the plates which contain ampicillin.

2. **What is the purpose of the control plates? Explain the difference between each and why it is necessary to run each.**

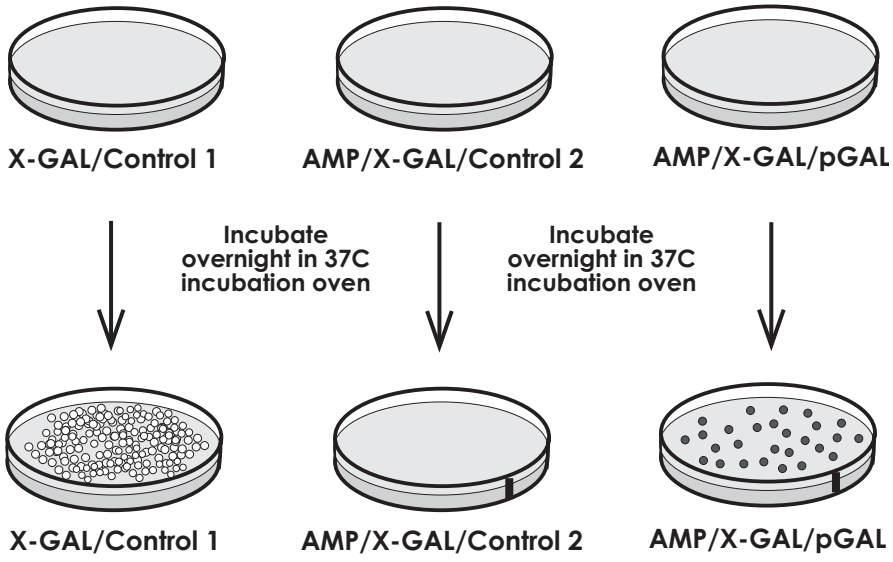
   Control plates help interpret the experimental results. There are two control plates in this experiment. The control plate that is labeled AMP/X-GAL/Control 2 shows that cells without the plasmid will not grown in the presence of ampicillin. The control plate X-GAL/Control 1 shows that host cells were not damaged during the transformation process and therefore are able to grow on agar plates that do not contain ampicillin.

3. **Why would one compare plates AMP/X-GAL and AMP/X-GAL/pGAL?**

   Cells not treated with the plasmid will not grow on the plate with ampicillin (AMP/X-GAL) because they are not expressing the ampicillin resistance gene. However, cells treated with the plasmid will grow on the AMP/X-GAL/pGAL plate because they are expressing the ampicillin resistance gene.

Experiment Results and Analysis

IDEALIZED SCHEMATIC OF RESULTS.



White colonies (Lawn of bacteria)

No colonies

Blue colonies

X-GAL plated with control cells (no DNA)

Result: Plate covered with white colonies. May look like a smeared layer or lawn of cells.

Demonstrates: Colonies are white because the cells do not utilize X-Gal. They do not contain pGAL DNA which contains a gene that will allow the cell to have a functional β-galactosidase.

AMP/X-GAL plated with control cells (no DNA)

Result: No growth

Demonstrates: Host cells are sensitive to ampicillin. Without pGAL DNA, they are not ampicillin-resistant. They do not make β-lactamase.

AMP/X-GAL plated with cells + DNA (pGAL)

Result: Blue colonies that may have white satellite colonies

Demonstrates: 1) A small portion of the cells are transformed; 2) Cells are transformed with pGAL and therefore can utilize X-GAL to give a blue color; 3) Cells acquire pGAL DNA and therefore ampicillin resistance.

Study Questions and Answers

1. **Did you observe any satellite colonies? Why are the satellite, feeder colonies white?**
   Not all cells become transformed. Some of the untransformed cells can grow around the colonies containing transformed cells. The transformed cells secrete β-lactamase which clears the surrounding medium of ampicillin. Therefore, some of the untransformed cells grow. The satellites are white since they did not incorporate pGAL DNA which contains the gene that will allow the cell to have a functional β-galactosidase.

2. **Why did the competent cells which did not receive DNA (control) fail to grow on the plates containing ampicillin?**
   Without pGAL DNA, they are not ampicillin-resistant because they do not make β-lactamase.

3. **Why are there so many cells growing on the X-GAL plate? What color are they?**
   Cells have not been challenged with antibiotic and therefore virtually all survive. They are white in color because they do not have pGAL DNA, and therefore, do not have a functional β-galactosidase.

4. **What evidence do you have that transformation was successful?**
   A successful transformation will show colonies on the plate labeled AMP/X-GAL/pGAL. An unsuccessful transformation will not show any colonies.

5. **What are some reasons why transformation may be unsuccessful?**
   Unsuccessful transformations could be the result of many things, including: 1) not adding the plasmid to the host cells in the + pGAL DNA tube, or 2) not adding a colony of bacteria to the + pGAL DNA tube, and 3) improper timing of the heat shock step.

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): Ampicillin

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: 07/01/03

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)
Ampicillin				
CAS# 7177-48-2	No data			

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 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: Slightly soluble

Appearance and Odor: Odorless, white crystaline powder

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: Dry chemical, carbon dioxide, water spray or regular foam

Special Fire Fighting Procedures: Move container from fire area if possible. Do not scatter spilled material with water streams.

Unusual Fire and Explosion Hazards: Avoid breathing vapors.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Incompatibles

Incompatibility: Strong oxidizers

Hazardous Decomposition or Byproducts: Toxic oxides of carbon, nitrogen and sulfur

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	Incompaticles

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Sensitizers may result in allergic reaction

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

Signs and Symptoms of Exposure: Repeated exposure may result in sensitization and possible anaphylactic shock.

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Ingestion: Allergic symptoms.
Eyes/Skin: Flush with water Inhalation: Move to fresh air

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing. Sweep up and place in suitable container for later disposal. Do not flush spilled material down sink.

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

Precautions to be Taken in Handling and Storing: Keep away from incompatible substances

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation				
	Local Exhaust	Yes	Special	None
	Mechanical (General)	No	Other	None

Protective Gloves: Yes Eye Protection: Splash or dust proof

Other Protective Clothing or Equipment: Eye wash

Work/Hygienic Practices: Wear protective clothing and equipment to prevent contact.

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): X-Gal in solvent

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: 02/08/05

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	at 760mm Hg	189C	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	20C	0.46	Melting Point	18C
Vapor Density (AIR = 1)		2.7	Evaporation Rate (Butyl Acetate = 1)	N/A

Solubility in Water: Complete (100%)

Appearance and Odor: Colorless liquid, faint odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
(closed cup) 88C (192F)		3%	43%

Extinguishing Media: Water spray, carbon dioxide, dry chemical, ordinary foam

Special Fire Fighting Procedures: Wear SCBA with full facepiece operated in positive pressure mode. If possible, move container from fire area

Unusual Fire and Explosion Hazards: Vapors may flow along surfaces to distant ignition sources and flashback. Closed containers exposed to heat may explode. Contact w/ strong oxidizers may cause fire.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Heat, flame, other sources of ignition

Incompatibility: Strong oxidizing agents, active halogen compounds, alkali metals

Hazardous Decomposition or Byproducts: Oxides of sulfur, mercaptane, active halogen compounds, alkali metals

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Chronic affects: kidney damage, liver damage

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

Signs and Symptoms of Exposure: Skin/eye irritation, headache, nausea, vomiting, dizziness

Medical Conditions Generally Aggravated by Exposure: Skin disorders

Emergency First Aid Procedures: Flush skin/eyes with large amounts of water. If ingested do not induce vomiting.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Shut off ignition sources. Wear protective clothing. Use water spray to reduce vapors. Take up with sand or other noncombustible absorbent material and dispose of properly

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

Precautions to be Taken in Handling and Storing: Store in cool, dry, well ventilated flammable liquid storage area or cabinet, store above 20C

Other Precautions: Product may solidify at room temperature

Section VIII - Control Measures

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

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

Protective Gloves: Butyl rubber gloves Eye Protection: Splash Proof Safety goggles

Other Protective Clothing or Equipment: Uniform or apron

Work/Hygienic Practices: Avoid contact with skin/eyes Keep container tightly closed.

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): Solvent for Induction of Competency

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: 05-25-05

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)
None	---------- Not established ----------			

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 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: Clear liquid

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Use water spray, alcohol foam, dry chemical, or carbon dioxide

Special Fire Fighting Procedures: Wear protective equipment and SCBA with full facepiece. Move container from fire area if possible.

Unusual Fire and Explosion Hazards: Vapors may flow along surfaces and flash back.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Avoid incompatibles
	Stable	X	

Incompatibility: Strong oxdizers

Hazardous Decomposition or Byproducts: Sulfur dioxide, mercaptans, carbon monoxide, carbon dioxide, formaldehyde

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	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/Ingestion: Nausea and vomiting
Skin/eye contact: Rapid absorption causing irritation

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

Signs and Symptoms of Exposure: Irritation

Medical Conditions Generally Aggravated by Exposure: Skin disorders

Emergency First Aid Procedures:
Ingestion: Call medical help, do not induce vomiting
Inhalation: remove to fresh air
Skin/eye contact: Flush w/ water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear protective clothing. Take up with sand or other absorbant and place in container
Dispose of properly.

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

Precautions to be Taken in Handling and Storing: Avoid contact

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): SCBA

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

Protective Gloves: Butyl rubber gloves

Eye Protection: Safety goggles

Other Protective Clothing or Equipment: Uniform or apron

Work/Hygienic Practices: Avoid contact