Biotechnology Basics™ by Ellyn Daugherty

Micropipetting

Measuring Very Small Volumes in Biotechnology

(Lab 3b)

(Cat. # BBED-3B)

Developed in partnership with

Micropipetting: Measuring Very Small Volumes in Biotechnology (Lab 3b)

Teacher's Guide

The following laboratory activity is adapted from "Laboratory 3b: Measuring Very Small Volumes In A Biotechnology Lab" from *Biotechnology: Science for the New Millennium Laboratory Manual* by Ellyn Daugherty. For more information about the program, please visit www.emcp.com/biotechnology. This kit is produced under license from Paradigm Publishing, Inc., a division of New Mountain Learning.

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About Ellyn Daugherty: Ellyn Daugherty is a veteran biotechnology educator and recipient of the Biotechnology Institute's National Biotechnology Teacher-Leader Award. She is the founder of the San Mateo Biotechnology Career Pathway (SMBCP). Started in 1993, SMBCP has instructed more than 10,000 high school and adult students. Annually, 30-40 SMBCP students complete internships with mentors at local biotechnology facilities.

About G-Biosciences: In addition to the Biotechnology by Ellyn Daugherty laboratory kit line and recognizing the significance and challenges of life sciences education, G-Biosciences has initiated the BioScience Excellence™ program. The program features hands-on teaching kits based on inquiry and curiosity that explore the fundamentals of life sciences and relate the techniques to the real world around us. The BioScience Excellence™ teaching tools will capture the imagination of young minds and deepen their understanding of various principles and techniques in biotechnology and improve their understanding of various social and ethical issues.

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Upon receipt, store the materials as directed in the package literature.

MATERIALS INCLUDED

This kit has enough materials and reagents for 8 lab groups (8 student pairs or 4 groups of 32 students).

- 8 vials of Red Dye, 2ml
- 8 vials of Green Dye, 2ml
- 16 vials of Blue Dye, 2ml
- 16 vials of Yellow Dye, 2ml
- 32 1.5-mL microcentrifuge tubes (4/group)
- 8 small foam microcentrifuge racks
- 16 8-well assay strips

ADDITIONAL EQUIPMENT & MATERIALS REQUIRED

- P1000, P200, P10 micropipette and tips
  These can be purchased separately

Cat. #	Fisher Sci Cat #	
BT1501	501059650	Pipette, Variable, P10
BT1502	501059651	Pipette, Variable, P100
BT1503	501059652	Pipette, Variable, P1000
BT1504	501059653	Pipette, Variable, P2
BT1505	501059654	Pipette, Variable, P20
BT1506	501059655	Pipette, Variable, P200

- Permanent marker, 8
- Paper towels
- Beaker or container for trash, 8

OPTIONAL MATERIALS:

- Balance or scale that measures to 0.001 g

SPECIAL HANDLING INSTRUCTIONS

- All components can be stored at room temperature until ready to use.
- Use paper towels as lab matting since dyes can permanently stain paper and cloth.

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GENERAL SAFETY PRECAUTIONS

- The reagents and components supplied in the Biotechnology Basics by Ellyn Daugherty™ kits are considered non-toxic and are safe to handle (unless otherwise noted), however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.
- The teacher should 1) be familiar with safety practices and regulations in his/her school (district and state) and 2) know what needs to be treated as hazardous waste and how to properly dispose of non-hazardous chemicals or biological material.
- Students should know where all emergency equipment (safety shower, eyewash station, fire extinguisher, fire blanket, first aid kit etc.) is located and be versed in general lab safety.
- Remind students to read all instructions including Safety Data Sheets (SDSs) before starting the lab activities. A link for SDSs for chemicals in this kit is posted at www.gbiosciences.com
- At the end of the lab, all laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. All used culture vials, strips, tips, or may be discarded in regular trash.

TEACHER'S PRE EXPERIMENT SET UP

1. Distribute the materials to each group:
    - 1 vial Red Dye
    - 2 vials Blue Dye
    - 2 vials Yellow Dye
    - 1 vial Green Dye
    - 4 1.5-mL microcentrifuge tubes
    - Small microcentrifuge rack
    - 2 8-well strips (350-µL volume wells)
    - A permanent lab marker
    - 1 piece of paper towel
    - Micropipettes and Micropipette tips
    - "TRASH" beaker or container, 8
2. If available, a balance or scale that measures to 0.001 g should be set up in a common area of the lab.

TIME REQUIRED

- 30 minutes for teacher preparation of stock solutions and distribution of reagents
- 1-hour lab period for micropipette use training and practice.
- 1-hour lab period for Micropipetting Challenge and Analysis

NEXT GENERATION SCIENCE STANDARDS ADDRESSED

- HS-LS1: From Molecules to Organisms: Structures and Processes
- LS1.A: Structure and Function

For more information about Next Generation Science Standards, visit: http://www.nextgenscience.org/

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EXPECTED RESULTS

Reflection/Analysis

Describe how well your team produced the 8-well sample. What technician errors may have been made? How might these be avoided in the future?

Response: If measured and dispensed correctly then the following should be true about the 8-well samples:

- Each cell's volume should be the same (350 µL) and should fill each well equally.
- The colors of the samples from left to right should be like a rainbow or spectrum with colors transitioning through violet, indigo, blue, green, yellow, orange and red.

Thinking Like a Biotechnician:

1. A technician in a lab thinks that the micropipette being used is not measuring correctly. Describe how to check the measuring accuracy of a pipette using a scale or balance.

   Answer: Answers will vary however each should include a discussion of how the technician can dispense know volumes onto weigh paper on a zeroed balance. The mass of a sample can be predicated because 1 ml (1000 µL) weighs 1 gram. So, 500 µL weighs 0.5 grams and 250 µL weighs 0.25 g, and so on.
2. For most experiments, several reagents must be added to the same tube and mixed without losing any sample. Propose a method to keep track of the samples that have been added to a reaction tube to create an homogenous mixture.

   Answer: Answers will vary however each should include a discussion of how the technician can check or cross off each sample on the procedure or protocol as it is being added. Each sample should be added to any sample already in the reaction tube to ensure mixing. When dispensing, the tip of a micropipette should only be submerged into a sample enough for mixing without losing any sample out of the tube by displacement.

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OBJECTIVES

After training and practice using the provided micropipettes, can you pipet small volumes within an acceptable amount of error?

BACKGROUND

Very tiny amounts of chemicals and biological reagents are used in many biotechnology experiments. To measure these minute volumes, technicians use micropipettes that measure microliter (µL) amounts. You are familiar with milliliter volumes. A microliter is 1/1000th of a milliliter. There are 1000 µL in 1 mL, so 1 µL = 0.001 mL.

That means 0.5 mL = 500 µL.

How many µL are there in 0.75 mL? ____

How many mL are 1,250 µL? ____

How much of a mL is 30 µL? ____

This activity introduces Micropipetting technique. As with all fine motor skills, learning how to use a micropipette takes practice and determination. You must be able to measure these very microliter volumes with accuracy. Operate the micropipette slowly and carefully. Micropipettes are expensive instruments and measuring accurately saves time and reagents and therefore increases productivity in a lab.

CAUTIONS

- NEVER rotate the volume adjuster beyond the upper or lower range of the pipette
- NEVER invert or lay the micropipette down with a filled tip; fluid can run back into the piston.
- NEVER let the plunger snap back after withdrawing or expelling fluid; this could damage the piston.
- NEVER immerse the barrel of the micropipette in fluid.
- NEVER reuse a tip that has been used to measure a different reagent.

Parts of a Micropipette

Each brand of micropipette is slightly different; however most micropipettes have the following parts.

Plunger

When you first pick up a micropipette look to see it's range of measurement, which is usually printed on the plunger or barrel of the micropipette. The range of measurement tells you the maximum and minimum volumes that the micropipette can measure. Do not try to measure volumes outside of the range of measurement.

The plunger is at the top. It gets pushed down to evacuate air from the internal chamber. Often the kind/size of micropipette is shown on the plunger. Some micropipettes show the pipet size on the side of the barrel.

Usually there are three sizes of micropipettes in the lab: a "P-20" (with a range of 2.0 to 20 µL), a "P-200" (with a range of 20-200 µL), and a "P-1000" (with a range of 200-1000 µL). Note: some laboratories have a "P-10" (for 0.5 to 10 µL) instead of a P-20 or a P-100 (for 10 to 100 µL) instead of a P-200.

What kind of micropipettes are at your lab station? _____

What are their ranges of measurement? _____

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Volume Display

The volume display is usually on the barrel side. It can be changed to show the volume of liquid to be measured. Before you change the volume setting on a micropipette be sure to check its range. Never turn the volume setting above or below it maximum or minimum volume.

To change the micropipette volume setting it depends on the brand of micropipette you are using. On some micropipettes the plunger is turned. On some micropipettes there are black dials that turned. On some micropipettes there is a locking button that must be depressed before the plunger or dial is turned. Check with your instructor to see how your micropipettes' volume is changed and describe the process below.

_____

_____

_____

_____

_____

_____

_____

Tip Holder

Solutions that are measured must not be sucked up into the inside on the micropipette barrel. To prevent this, a disposable tip is placed at the end of the micropipette prior to each use.

Usually there are a few different sizes of micropipette tips in a lab. Make sure you have the right one for your micropipette. A P-1000 usually uses a blue or white tip. A P-200, P-100, or P-20 usually uses yellow or white tips. A P-10 usually uses white tips.
NOTE: *If a tip fits correctly it stays on the micropipette snuggly and measures accurately for several pipettings.*

Disposable tips are usually available in tip trays (often with color coding to match the tips) and are added to the micropipette by gently pressing the micropipette onto the tip. Press firmly but not so hard that the micropipette bends or gets damaged.

Tip Ejector

A tip ejector is usually found at the top of the pipet next to the plunger. By depressing the ejector the tip is ejected into a trash receptacle without the technician having to touch the tip itself.

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MATERIALS FOR EACH GROUP

Each group should have the following components from the kit:

- 1 vial Red Dye
- 2 vials Blue Dye
- 2 vials Yellow Dye
- 1 vial Green Dye
- 4 1.5-mL microcentrifuge tubes
- Small foam microcentrifuge rack
- 2 8-well strips (350-µL volume wells)

ADDITIONAL MATERIALS FOR EACH GROUP

The following standard lab equipment should be available for each group.

- Permanent lab markers, 8
- Paper towels
- Micropipettes and Micropipette tips
- Balance or scale that measures to 0.001 g (optional)
- "TRASH" beaker or container, 8

PROCEDURE

1. Assign a different dye solution to each lab partner for Micropipetting practice. This will allow for quick confirmation of Micropipetting skill.

Part I: Use of a P-200 or P-100

1. Check with you partner that you have the right micropipette (a P-200 or P-100). If you are unsure, reread the Background section or check with your instructor.

2. Dial the desired volume of 75 µL. Do you understand how to read the scale? If not, ASK or review the background information. Confirm with your group members that the volume is set correctly.

3. Push the end of the pipet into the proper size tip. The yellow tips or white tips in yellow trays are usually used with P-200s and P-100s.

4. Before using the micropipette, push down on the plunger to feel the "first stop." Then push farther on the plunger to feel the "second stop." The 1st stop is to evacuate air of the volume shown on the display. The second stop pushes out the 1st stop volume plus about 50% more air to make sure all of a measure sampled is evacuated from the tip. Repeat several times until you are certain you feel the difference between the 1st and 2nd stops. Pass the micropipette to other group members and have them do the same.

5. Set up and label 1 microcentrifuge tube for each group member (with an initial) in the microcentrifuge tube rack. Each group member will dispense 75 µL of their assigned colored solution to their labeled tube by holding the micropipette in one hand, almost vertically, holding the opened, colored vial in the other hand. Both should be at almost eye-level.

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6. The user will depress the plunger of the micropipette to the first stop, and hold it in this position, then dip the tip into the solution to be pipetted to a depth of 2-5mm. Slowly raise the plunger to draw fluid into the tip. Keep micropipette in a vertical (or close to vertical) position. Cap the vial.

7. To dispense the sample from the micropipette, hold the micropipette in one hand, almost vertically. Hold the centrifuge tube in your other hand. Both should be at about eye-level. Gently touch the micropipette tip to the inside wall of the appropriate centrifuge tube and slowly depress the plunger of the micropipette all the way to the 2nd stop. Make sure the tiny volume is dispensed to the bottom of the tube. Hold the plunger down as you draw the micropipette out of the tube to not suck back up any of the sample. Close the microcentrifuge tube tightly. Eject the used tip into the trash.

8. Each group member should repeat the measurement with their assigned color solution. Make sure to use a fresh tip for measurement on each micropipette that is used.

9. Hold up each dispensed sample with the closed caps of the tubes aligned. If they are all the same volume, the top of the samples (meniscus) should touch. If any sample is stuck on the side of the tube, give the closed tube a swift wrist-flick to pool all the sample at the bottom of the tube.

10. To make sure that the volume in each tube is correct (75 µL), a balance, if available, may be used to measure the sample in a closed tube.

11. Weigh an empty microcentrifuge tube. Weigh the centrifuge tube with the 75 µL sample. Subtract the empty tube's weight from the sample + tube's weight (this gives you the weight of the liquid in the tube. Since 1 mL of water weighs 1 g, then 1000µL of water weighs 1 g (or 1000 mg) and 75 µL weighs 0.075 g or 75 mg.
If that liquid volume weight is 0.075 g +/- 0.01 grams, consider yourself a good micropipetter and record in your notebook that you accomplished the P-200 or P-100 skill development.

12. Use the micropipette to completely transfer all the solution in your tube to the trash. Your empty tube will be reused for Part II.

Part II: Use of a P-1000

1. Each group member should repeat Part I, Steps 1-9, using a P-1000 to dispense 330 µL. Be careful to pipet slowly and watch to not add air spaces or bubbles to the sample.

2. Confirm with each group member that the volume display is being read correctly before starting and make sure to use the appropriate tips (white or blue) with the P-1000. Use the same colored stock solutions and emptied labeled tubes for each group member from Part I.

3. After each group member has measured their samples compare them to each other by aligning the samples (Part I, Step 9) and weighing the sample (Part I, Step 10-11, except that the fluid volume should weigh 0.330 g).

4. If that liquid volume weight is 0.330 g +/- 0.03 grams, consider yourself a good micropipetter and record in your notebook that you accomplished the P-1000 skill development.

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5. Use the micropipette to completely transfer all the solution in your tube to the trash. Your empty tube will be reused for Part III.

Part III: Use of a P-20 or P-10

1. Each group member should repeat Part I, Steps 1-9, using a P-20 or P-10 to dispense 8 µL.

2. Confirm with each group member that the volume display is being read correctly before starting and make sure to use the appropriate tips (white or grey) with the P-20 or P-10. Use the same colored stock solutions and emptied labeled tubes for each group member from Part II. Make sure the tiny volume is dispensed to the bottom of the tube.

3. After each group member has measured their samples compare them to each other by aligning the samples (Part I, Step 9) and weighing the sample (Part I, Step 10-11, except that the fluid volume should weigh 0.008 g).

4. If that liquid volume weight is 0.008 g +/- 0.001 grams, consider yourself a good micropipetter and record in your notebook that you accomplished the P-20 or P-10 skill development.

5. Use the micropipette to completely transfer all the solution in your tube to the trash. Your empty tube will be reused for Part IV.

Part IV: Practice Dispensing Different Samples into the Same Reaction Tube

1. Using the tubes used in Part III, assign each group member a number either 1, 2, 3, or 4. Each person should label the microcentrifuge tube with their number and their initial.

2. Each group member will micropipette the designated stock samples shown on the Reaction Matrix into their labeled tubes.

3. Group members must share pipettes, changing the volumes as necessary. Make sure that all volumes dispensed to the bottom of the tube. Before removing the tip from the final dispensing, give the sample of gentle swirl to mix the colors.

Remember:

- Use the smallest instrument possible for all measurements.
- Change tips every time.
- If more than one solution is dispensed into a tube, pipet each volume into the sample already in the tube.
- Total volumes may be checked by setting a P-1000 to the total volume and sucking the entire volume into the tip. If no sample remains in the tube and there are no air spaces in the tip, the total volume was measured correctly.
- Evaluation criteria: volumes, final colors

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Reaction Matrix I for Practice Samples

	Tube 1	Tube 2	Tube 3	Tube 4
Red Dye (µL)	44	0	17	0
Blue Dye (µL)	165	0	0	146
Green Dye (µL)	0	28	125	0
Yellow Dye (µL)	0	119	0	22
Total Volume (µL)	209	147	142	168

4. After all group members have produced their practice tubes, find other groups in the lab to compare your samples. Look to see that the meniscus of similar samples line up. If any sample seems to be inaccurately measured, check it using a balance or use a 3rd groups' samples to check. If any sample's volume is obviously incorrect, discard the volume and prepare the sample again.

5. If the meniscus of your sample matches with others in the class, record in your notebook that you have mastered pipetting multiple samples.

6. Use the micropipette to completely transfer all the solution in your tube to the trash. Your empty tube will be reused to pipet the samples for Reaction Matrix II.

7. Repeat Part IV using the Reaction Matrix II below

Reaction Matrix II for Practice Samples

	Tube 1	Tube 2	Tube 3	Tube 4
Red Dye (µL)	4	310	12	15
Blue Dye (µL)	65	17	212	6
Green Dye (µL)	368	8	7	72
Yellow Dye (µL)	14	79	188	435
Total Volume (µL)	451	414	419	528

Part V: Micropipetting Challenge (for a 2 person team)

1. Place a 8-well test strip on your lab table oriented with the strip laid out from left to right. Using a lab marker pen, put your initials on the side of the back side of the left most well and then label the wells from 1 -8 on the front side. Make the labels small so that the color of the marker does not block the view of the wells from the side.
2. Each team pipets the following volumes of the designated solutions into the assigned wells.

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Cell	1	2	3	4	5	6	7	8
Yellow Dye (µL)	0	0	0	175	350	325	240	75
Red Dye (µL)	175	85	0	0	0	25	110	275
Blue Dye (µL)	175	265	350	175	0	0	0	0

REFLECTION/ANALYSIS

Evaluate your team's Micropipetting skills. If you measured and dispensed correctly then the following should be true:

Each cell's volume should be the same (350 µL) and should fill each well equally.

The colors of the samples from left to right should be like a rainbow or spectrum from dark blue to light blue to green to red, then orange and finally yellow.

Describe how well your team produced the 8-well sample. What technician errors may have been made? How might these be avoided in the future?

THINKING LIKE A BIOTECHNICIAN:

1. A technician in a lab thinks that the micropipette being used is not measuring correctly. Describe how to check the measuring accuracy of a pipet using a scale or balance.
2. For most experiments, several reagents must be added to the same tube and mixed without losing any sample. Propose a method to keep track of the samples that have been added to a reaction tube to create an homogenous mixture.

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