

How to Use a Micropipettor

Introduction

One important tool in biotechnology is the micropipette, which accurately measures and dispenses small volumes of liquid. Although micropipettors are precise tools, their accuracy is very dependent on the skill of the user. The accurate use of the micropipettor requires instruction and practice.

Practice using the push-button:

- The **first stop** is used for picking up the liquid.
- The **second stop** is used for dispensing.
- Place a tip firmly on the end of the micropipettor.
- Practice picking up various volumes of liquid. Look at the liquid in the tip so that you know about how much of the tip is filled by different volumes.

Good technique requires consistency:

- Consistent pickup and dispense rhythm.
- Consistent speed when you press and release the push-button. Try to have a very smooth action.
- Consistent push-button pressure at the first stop.
- Consistent immersion depth. Do not place the tip directly on the bottom of the tube.
- Minimal angle (<20° from vertical).

When you think you have mastered the skills required for accurate use of the micropipettor, do the following exercise. You will measure different volumes of a concentrated dye (Fast Green at 0.5 $\mu\text{g}/\mu\text{l}$) into a cuvette with water and then quantitate the actual amount of dye in the tube using a spectrophotometer. If your pipetting skills are good, a graph of the amount of Fast Green versus the absorbance in the spectrophotometer should give a straight line.

Materials (per student or group)

- a tube of Fast Green dye (0.5 $\mu\text{g}/\mu\text{l}$)
- 11 plastic cuvettes for spectrophotometer
- micropipettor (1-20 μl)
- micropipettor (100-1000 μl)
- micropipettor tips for each size micropipettor
- water
- Parafilm
- access to a spectrophotometer

In the chart below:

1. Calculate the amount of water needed to give a final volume of 1.0 ml (1,000 μl) and write that value in column 2.
2. Calculate the μg of Fast Green in each sample and write that value in column 4 and again in column 1 of Table 2.

Table 1.

Volume of Fast Green (0.5 $\mu\text{g}/\mu\text{l}$)	Volume of water needed (μl)	Total volume	μg of Fast Green in cuvette
0 μl	1,000 μl	1.0 ml	0
1 μl		1.0 ml	
2 μl		1.0 ml	
5 μl		1.0 ml	
10 μl		1.0 ml	
20 μl		1.0 ml	

Procedure

1. Using the appropriate size micropipettor add the volume of Fast Green shown in column 1 of the table above to each of two 1.6 ml plastic cuvettes. These are duplicates that should be labeled "A" and "B". You should have 10 cuvettes, two for each amount of Fast Green.
2. In another cuvette place 1.0 ml water to serve as the blank for the spectrophotometer.
3. Using the appropriate size micropipettor (different from the one used in step 1) add the volume of water shown in column 2 in the table above to the appropriate cuvettes.
4. Place a piece of Parafilm over each cuvette and invert 3-4 times to mix thoroughly.
5. Read the absorbance of each sample at 620 nm. Use the cuvette with water as the blank to set the spectrophotometer to zero.

Table 2.

μg of Fast Green	A_{620} reading of Sample A	A_{620} reading of Sample B	Mean value for two samples	Standard deviation for two samples
0	0	0	0	

6. Graph the data from the Table above using the μg of Fast Green on the x-axis and the mean of the A_{620} for the two samples on the y-axis. Connect the data points.

Results

1. If your technique with the micropipettor was good, the graph you produced should be a straight line. The more points that deviate from a line, the more inaccurate the pipetting skill.
2. The standard deviation for the two values for the duplicate tubes provides a measure of the reproducibility of your pipetting skills. The smaller the standard deviation, the better your skills.

Teacher Preparation

Prepare Fast Green dye at a final concentration of 0.5 $\mu\text{g}/\mu\text{l}$ and aliquot into small tubes for each student.

Turn on the spectrophotometer and set it to 620 nm.

Demonstrate the correct use of the micropipettors to the students and have them practice using the micropipettors with water before they perform the lab exercise.

Contributed by

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