

APPENDIX I. Pipettors and Pipetting

We will be using two types of pipettes: the Rainin Pipetman adjustable digital pipette and a traditional glass serological pipette. The general operating procedure of each type follows.

1. Pipetman

Used to accurately and precisely transfer liquid samples from one place to another.

The digital volume indicator in the pipette shaft is read from top to bottom (the top being where your thumb goes). P-20 and P-200, black digits indicate microliters and red digits tenths of microliters. For P-1000 red digits indicate milliliters and black digits microliters.

Sample values and volume ranges for each Pipetman model are shown below:

	P-20	P-200	P-1000
Top	1	1	0
Middle	2	2	7
Bottom	5	5	5
	12.5 mcL	125 mcL	750 mcL

Model	Recommended Range (mcL)
P-20	2-20
P-200	20-200
P-1000	100-1000

Operation

There are two ways of setting volume on Pipetman pipettes: with the volume adjustment knob, or by turning the plunger button. Both give exactly the same results.

Volume Setting using Plunger Button

1a. Hold Pipetman in one hand. With thumb and forefinger, turn the plunger button counterclockwise until the volume indicator is 1/3 revolution above the desired setting, then turn slowly clockwise until the desired volume shows on the indicator.

Volume Setting using Volume Adjustment Knob

1b. Hold Pipetman in one hand. With the other hand, turn the volume adjustment knob counterclockwise so the volume indicator is 1/3 revolution above the desired setting, then turn slowly clockwise until the indicator shows the desired volume.

2. ALWAYS dial down to the desired volume. This prevents mechanical backlash from affecting accuracy. If you pass the desired setting, turn the dial 1/3 revolution higher than desired and reset the volume. The friction ring prevents unintentional volume changes.

3. Attach a new disposable tip to the pipette shaft. Press only enough to make a positive airtight seal.
 4. Press the plunger to the FIRST STOP. This part of the stroke is the volume displayed on the indicator.
 5. Holding Pipetman vertically, immerse the tip into the sample
 6. Allow the pushbutton to return slowly to the UP position. Never let it snap up!
 7. Pause briefly to ensure that the full volume of sample is drawn into the tip.
 8. Withdraw the tip from the sample liquid.
 9. To dispense sample, touch the tip end against the side wall (or below the surface of the liquid) of the receiving vessel and depress the plunger slowly to the first stop.
Wait: 1 second* (P-2, P-10, P-20, P-100, P-200)
1-2 seconds* (P-1000)
* Longer for viscous solutions.
- Then press the plunger to the SECOND STOP (bottom of stroke), expelling any residual liquid in the tip.
10. With the plunger fully pressed, withdraw Pipetman from the vessel carefully, tip against the vessel wall.
 11. Allow the plunger to return to the up position.
 12. Discard the tip by depressing the tip ejector button. A fresh tip should be used for each sample to prevent sample carryover.

Pipetting Guidelines & Precautions

Consistency in all aspects of pipetting procedure will significantly contribute to optimum reproducibility. Use a:

1. Consistent pickup/dispense rhythm while pipetting.
2. Consistent speed and smoothness when you press and release the pushbutton.
3. Consistent pushbutton pressure at the first stop.
4. Consistent immersion depth.
5. Minimal angle (< 20° from vertical).

2. Serological pipettes

Generally used with larger volumes and when accuracy and precision are not as important.

For example

- measuring the milliliter amounts of solutions for a 6% acrylamide gel
- adding lysis buffer to a bacterial pellet
- transferring viscous material to centrifuge tubes