

Pipetting skills

How are small volumes of liquid measured?

Background

Micropipettes are used to transfer small measured volumes of liquid in the lab. Micropipettes can be fixed rate or adjustable, and can vary in the increment measured as well, full μl or fractions of a μl . On an adjustable micropipette, the volume can be changed by twisting the handle or top of the plunger, depending on the brand/type you have. The readout dial will show the volume the micropipette is adjusted to transfer. The micropipette is limited to the volume range that is pre-set on the instrument. The most common ranges include: 0.5–10 μl , 2–20 μl , 10–100 μl and 100–1,000 μl . A pipette tip must be used with the micropipetter. The tip is where the liquid is stored as it is being transferred. A new pipette tip should be used for each different liquid. The tip is ejected by using the ejector mechanism on the micropipette; some tips must be manually removed, depending on the micropipette.

When ready to draw up a liquid, obtain a clean tip. Press the pipette into the open end of the tip and tap gently to “seat” the tip on the pipette. Place the tip in the liquid, just below the level of the liquid, in the center of the sample, but not to the base of the container. Depress the plunger on the pipette to the “soft” or first stop. The position of this stop will vary depending on the volume set to transfer (since the point of initial resistance is determined by the desired volume of solution being transferred). Release the plunger to draw up the liquid. Once the desired volume is drawn into the tip, remove the pipette, and take the liquid to its destination. Release the liquid by depressing the plunger, first to the “soft” stop, then one second later to the second or “hard” stop to remove all of the contents. Remove the tip from the liquid before the plunger is released. Discard the tip by ejecting into a proper waste receptacle. Optional: watch the pipetting video: [youtube.com/watch?v=bex0itUMxml](https://www.youtube.com/watch?v=bex0itUMxml)

Before pipetting, practice!

Practice feeling the “soft” stop several times; taking the sample from the center of the container holding the liquid to be transferred; transferring the liquid while the pipette is upright; releasing the liquid into the appropriate microtube or cell well by touching the tip to the side and depressing the plunger, first to the “soft” stop, next to the “hard” stop; changing tips for each liquid.

Materials

- Micropipettors of various sizes
- 96-well plate per group
- Colored water in 10ml amounts (color needed dependent upon which protocol)
- Micropipetting protocol colors:
 - a. Red and Blue water
 - b. Yellow and Green water
 - c. Green and Blue water
 - d. Blue, Red and Green water
 - e. Red and Yellow water
 - f. Yellow and Green water
 - g. Blue and Red water

Instructions

Follow one of the protocols to practice pipetting. Each protocol can be passed out to an individual group.

Digital extra

Fisher Scientific pipetting lesson: [youtube.com/watch?v=bexOitUMxml](https://www.youtube.com/watch?v=bexOitUMxml)

Assessment

1. Have students check their accuracy by using a mass scale that measures to the thousandths.
 - a. Add up the volume of the liquid they added.
 - b. Convert volume to mass ($1000\mu\text{l} = 1\text{ml}$; $1\text{ml} = 1\text{g}$)
 - c. Zero a scale with and empty 96-well plate
 - d. Calculate the mass of their completed design
2. Did the group follow directions and figure out the letters they pipetted?

Answers

A = DNA = $5045\mu\text{l}$

B = Corn = $5841\mu\text{l}$

C = pGLO = $5217\mu\text{l}$

D = a plasmid = $1932\mu\text{l}$

E = GMO = $4694\mu\text{l}$

F = KCC = $4104\mu\text{l}$

G = DDG = $4800\mu\text{l}$

Protocol A

Micropipette the indicated volumes into designated wells on the 96 well plate.

USING THE RED WATER

- 20 μ l: B1, B2, B3, B11
- 26 μ l: D1, D3, D10, D11, D12
- 27 μ l: E1, E3, E10, E12
- 28 μ l: F1, F2, F10, F12
- 29 μ l: C1, C3, C10, C12

USING THE BLUE WATER

- 38 μ l: B5, B8
- 36 μ l: D5, D7, D8
- 48 μ l: E5, E7, E8
- 59 μ l: F5, F8
- 67 μ l: C5, C6, C8

USING THE RED WATER

- 90 μ l: B1, B2, B3, B11
- 132 μ l: D1, D3, D10, D11, D12
- 127 μ l: E1, E3, E10, E12
- 103 μ l: F1, F2, F10, F12
- 112 μ l: C1, C3, C10, C12

USING THE BLUE WATER

- 126 μ l: B5, B8
- 102 μ l: D5, D7, D8
- 96 μ l: E5, E7, E8
- 138 μ l: F5, F8
- 114 μ l: C5, C6, C8

Protocol B

Micropipette the indicated volumes into designated wells on the 96 well plate.

USING THE YELLOW WATER

20µl: E5, E6, E9, E12
27µl: F4, F7, F9, F10, F12
45µl: G4, G7, G9, G11, G12
38µl: H5, H6, H9, H12

USING THE GREEN WATER

119µl: A2, A3, A7, A8
48µl: B1, B4, B7, B9
67µl: C1, C7, C8
76µl: D2, D3, D4, D7, D9
55µl: E2, E3, E4

USING THE YELLOW WATER

70µl: E5, E6, E9, E12
110µl: F4, F7, F9, F10, F12
116µl: G4, G7, G9, G11, G12
93µl: H5, H6, H9, H12

USING THE GREEN WATER

129µl: A2, A3, A7, A8
118µl: B1, B4, B7, B9
107µl: C1, C7, C8
96µl: D2, D3, D4, D7, D9
88µl: E2, E3, E4

Protocol C

Micropipette the indicated volumes into designated wells on the 96 well plate.

USING GREEN WATER

20µl: B7, C7
47µl: D1, D2, D7
28µl: E1, E3, E7
59µl: F1, F2, F7, F8, F9
35µl: G1, H1

USING BLUE WATER

28µl: B5, B11
62µl: C4, C6, C10, C12
51µl: D4, D10, D12
90µl: E4, E5, E6, E10, E12
79µl: F5, F11

USING GREEN WATER

70µl: B7, C7
116µl: D1, D2, D7
110µl: E1, E3, E7
85µl: F1, F2, F7, F8, F9
93µl: G1, H1

USING BLUE WATER

118µl: B5, B11
96µl: C4, C6, C10, C12
88µl: D4, D10, D12
199µl: E4, E5, E6, E10, E12
107µl: F5, F11

Protocol D

Micropipette the indicated volumes into designated wells on the 96 well plate.

USING BLUE WATER

- 20µl: A6, A7
- 39µl: B5
- 28µl: C4, D4, E4
- 42µl: F5
- 51µl: G6, G7

USING RED WATER

- 100µl: A8
 - 111µl: B9
 - 120µl: C10, D10
- ### USING GREEN WATER
- 140µl: E10
 - 176µl: F9
 - 115µl: G8

USING BLUE WATER

- 70µl: A6, A7
- 81µl: B5
- 76µl: C4, D4, E4
- 92µl: F5
- 101µl: G6, G7

Protocol E

Micropipette the indicated volumes into designated wells on the 96 well plate.

USING RED WATER

- 20µl: B2, B3, B4, B11
- 35µl: C1, C10, C12
- 47µl: D1, D3, D4, D10, D12
- 28µl: E1, E4, E10, E12
- 59µl: F2, F3, F11

USING YELLOW WATER

- 88µl: B6, B8
- 26µl: C6, C7, C8
- 45µl: D6, D8
- 79µl: E6, E8
- 27µl: F6, F8

USING RED WATER

- 70µl: B2, B3, B4, B11
- 116µl: C1, C10, C12
- 110µl: D1, D3, D4, D10, D12
- 135µl: E1, E4, E10, E12
- 179µl: F2, F3, F11

USING YELLOW WATER

- 118µl: B6, B8
- 96µl: C6, C7, C8
- 89µl: D6, D8
- 129µl: E6, E8
- 107µl: F6, F8

Protocol F

Micropipette the indicated volumes into designated wells on the 96 well plate.

USING GREEN WATER

22µl: B1, B3, B10
31µl: C1, C2, C9, C11
47µl: D1, D9
29µl: E 1, E2, E 9, E11
40µl: F1, F3, F10

USING YELLOW WATER

32µl: B6, F6
23µl: C5, C7
74µl: D5, E6, E7
122µl: B1, B3, B10
131µl: C1, C2, C9, C11
147µl: D1, D9
129µl: E1, E2, E9, E11
140µl: F1, F3, F10

USING YELLOW WATER

162µl: B6, F6
143µl: C5, C7
174µl: D5, E6, E7

Protocol G

Micropipette the indicated volumes into designated wells on the 96 well plate.

USING BLUE WATER

119µl: B1, B2, B10, B11, B12
102µl: C1, C3, C9
127µl: D1, D3, D9, D11, D12
115µl: E1, E3, E9, E12
132µl: F1, F2, F10, F11

USING RED WATER

20µl: B5, B6
34µl: C5, C7
89µl: D5, D7
44µl: E5, E7
100µl: F5, F6

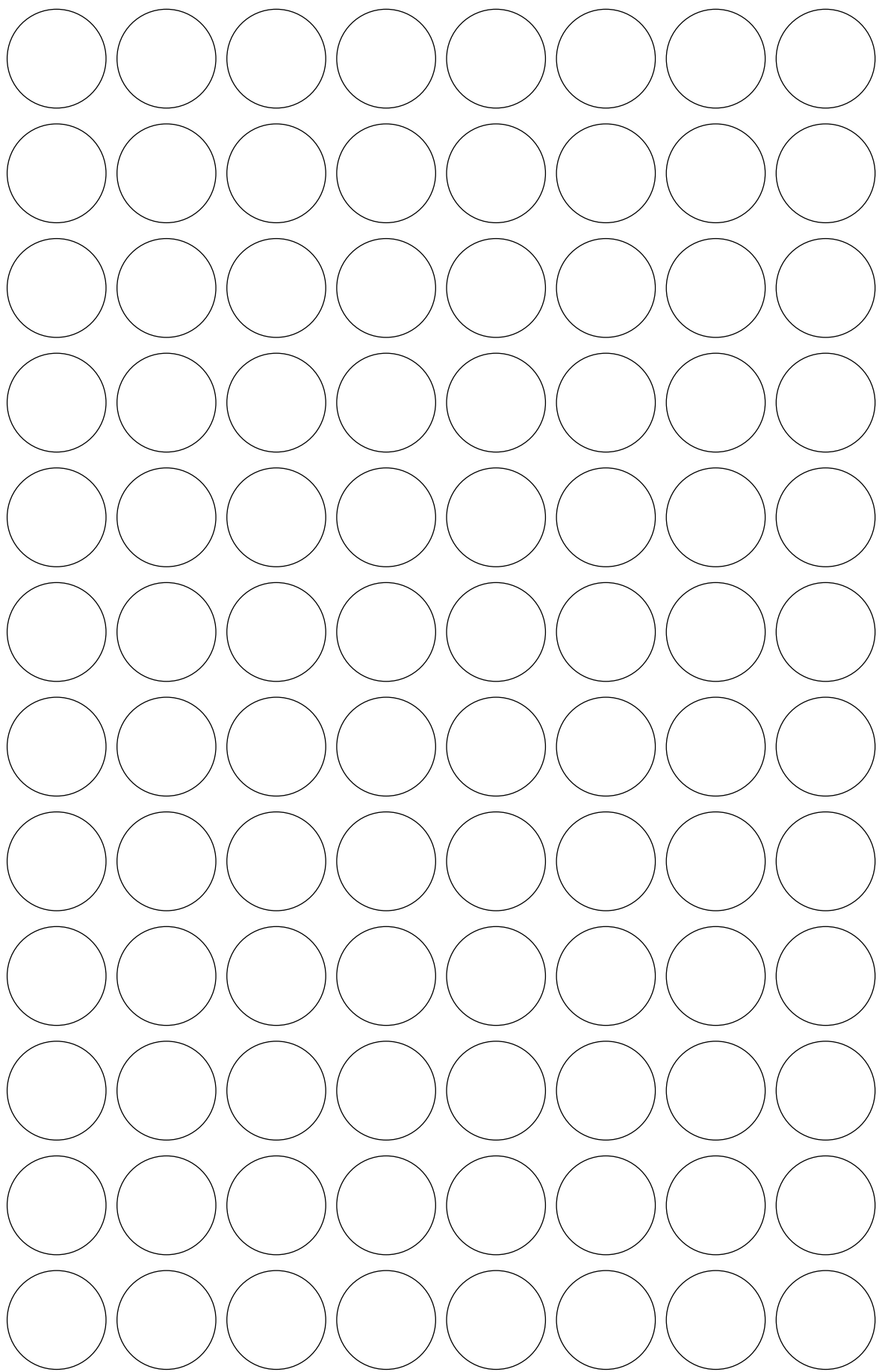
USING BLUE WATER

22µl: B1, B2, B10, B11, B12
31µl: C1, C3, C9
29µl: D1, D3, D9, D11, D12
22µl: E1, E3, E9, E12
40µl: F1, F2, F10, F11

USING RED WATER

74µl: B5, B6
158µl: C5, C7
37µl: D5, D7
196µl: E5, E7
88µl: F5, F6

1 2 3 4 5 6 7 8 9 10 11 12



A

B

C

D

E

F

G

H