Feb 21, 2021

Determination of the accuracy of micropipettes for the biochemistry lab

Chris Berndsen[1]

[1]James Madison University

In Development This protocol is published without a DOI.


Chris Berndsen
James Madison University

SUBMIT TO PLOS ONE

ABSTRACT

Basic protocol to help students use the laboratory micropipettes in the range of 1 to 1000 µL.

PROTOCOL CITATION

Chris Berndsen 2021. Determination of the accuracy of micropipettes for the biochemistry lab. **protocols.io** https://protocols.io/view/determination-of-the-accuracy-of-micropipettes-for-ba3digi6

KEYWORDS

pipette

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jan 07, 2020

LAST MODIFIED

Feb 21, 2021

PROTOCOL INTEGER ID

31557

GUIDELINES

This protocol is based on information from the Gilson Guide to Pipetting, 3rd Edition (2015).

MATERIALS TEXT

water
thermometer
micropipette
balance
pipette tips
computer
beaker
weigh boats

BEFORE STARTING

To start make sure that the balance you are using is calibrated and capable of measuring masses into the milligram range. If the balance is not capable of this level of sensitivity, then this protocol may not be appropriate for your work.

Before calibration

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

1 **20 mL** Fill a beaker with 20 mL of ddH_2O.

The volume does not have to be accurate.

2 Measure and record the temperature of the water.

3 Tare the balance with the weigh boat.

Measuring liquid dispensing

4 Set the volume of the pipette as shown in Step 4.1 to the appropriate volume for the measurement.

It is best to "dial down" to the desired volume, so you may need to go past your target volume and then dial back.

4.1

A	B	C	D
micropipette range	**low volume (uL)**	**middle volume (uL)**	**high volume (uL)**
1 to 10 uL	1	5	10
2 to 20 uL	2	10	20
1 to 100 uL	1	50	100
20 to 200 uL	20	100	200
100 to 1000	100	500	1000

Calibration volumes for different dispensing ranges of micropipettes

5 Put tip onto pipette by taking micropipette, placing the shaft into the desired tip and pushing while twisting slightly.

You do not need to hammer or slam the micropipettor into the tip to get it to stay.

Always use tips on micropipettors!

6 Depress the plunger on the micropipette to the first stop.

It is worth taking time to familiarize your self with the micropipette plunger positions if you have not used one before/recently. The first stop refers to the initial resistance of the plunger but is not fully depressed. The second stop is when the plunger is fully depressed. The up position is the default position.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

7 Submerge the pipette tip in the water about 2 mm below the menicus for small volume micropipettes (<20 µL) and 4 to 6 mm for larger volume micropipettes.

8 Allow the plunger to go to the up position. Depress the plunger to the first stop to eject the liquid, then allow the plunger to return to the up position. This is called "pre-rinsing".

The pre-rinse liquid can be ejected to waste as well if desired or to avoid contamination. In general, pre-rinsing should be down with each new tip or volume.

9 Remove pipette tip from water.

10 Eject water onto the weigh boat by depressing the plunger down to the second stop.

11 Remove tip from the liquid and then allow plunger to move to the up position.

12 Record the mass of the water.

13 Repeat steps 6 to 12, 5 times additional times and record the masses (6 total measurements).

14 Measure the temperature of the water to ensure it has not changed significantly during the measurements.

Determining accuracy of the pipette

15

Transfer your data into the calibration worksheet.

micropipette_calibration_worksheet.xlsx

15.1 Put the date, the name of your group, and the balance identity in the first row in the appropriate cells.

15.2 Fill in the temperature and the density of water in row 2.

In row 4, replace the text or fill in the cell next to pipette number for all three boxes.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

15.3 If you are only doing one pipette, these will all be the same. If you are testing different pipettes, this information can be different.

15.4 In rows 7 to 12 of column A, put in the volume in µL and in column B record the mass in milligrams.

1 mg is 0.001 g.

16 Repeat step 15 and all sub-steps for other volumes. Make note of any changes in accuracy across the pipette range. In step 15.4, you will use columns D/E, G/H, J/K, etc. to calculate the accuracy of the other volumes tested.

17 Upload data to the appropriate space on the Open Science Framework and move onto the next pipette.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited