Jul 24, 2024

Assessing Precision of One's Own Pipetting

DOI
dx.doi.org/10.17504/protocols.io.e6nvw1okzlmk/v1

Jonathan Phillips[1], Gregor Blaha[1]

[1]University of California, Riverside

G Gregor Blaha
University of California, Riverside

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.e6nvw1okzlmk/v1

Protocol Citation: Jonathan Phillips, Gregor Blaha 2024. Assessing Precision of One's Own Pipetting. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvw1okzlmk/v1

Protocol status: Working
We use this protocol and it's working

Created: July 21, 2024

Last Modified: July 24, 2024

Protocol Integer ID: 103786

Keywords: reproducibility, qPCR, in vitro assay, precision

Funders Acknowledgement:
California Department of Food and Agriculture
Grant ID: 21-0001-056-SF

Abstract

Accurate pipetting is critical for producing precise in vitro assay results. However, since pipetting is such a routine task, many researchers believe their pipetting is precise enough. Rarely do they critically evaluate their own pipetting. This protocol provides an affordable way to assess one's own pipetting.

Attachments

PDF

01 assessing_precise...

759KB

Image Attribution

For permission to use the image or to contact the creator of the image, irod, please contact the lead author at gregor.blaha@ucr.edu

Materials

Part A:
Micropipettes with adjustable volume of 100 – 1000 µL and 20 – 200 µL (ideally recently calibrated).

Pipette tips recommended by manufacturer of micropipettes.

Analytical balance with 0.1 mg or better precision.
For developing this SOP, an A&D HR-60 analytical balance was used. The balance was placed on a workbench in a low-traffic area and away from any drafts created by heating, ventilation, and air conditioning (HVAC). To minimize vibrations, the balance rested on a marble slab. The balance was leveled using the level bubble on the back of the balance. The air humidity in the area was increased using a humidifier. In addition, a water-soaked 3.5” x 5.5” x 0.5” sponge sitting in a water-filled reservoir was placed inside the balance’s draft shield.

Medium-sized antistatic weighing boat.

Water at room temperature.

Part B:
Spectrophotometer

5 and 10 mL volumetric or serological pipette with less than 0.2% error.

Electronic pipette aid

4 mL transfer pipettes with elongated stem (Fisher cat.: 501960843)

1 bag of hundred 5 mL centrifuge tubes (Eppendorf cat.: 003011401)

$K_3[Fe(CN)_6]$ (VWR cat.: TS22311-100)

100% [v/v] glycerol

Six 50 mL conical centrifuge tubes

50 mL volumetric flask

Vortex (optional)

Protocol references

Micropipette Manufacturers' webpages.

NIST 2012: NIST/SEMATECH e-Handbook of Statistical Methods,
https://www.itl.nist.gov/div898/handbook/eda/section3/eda358.htm
last retrieved 09/06/2023

Nolan T, et al. (2006) Quantification of mRNA using real-time RT-PCR. Nat Protoc. 1, 1559-82. PMID: 17406449. doi: 10.1038/nprot.2006.236.

de Ronde, M.W.J et al. (2017) Practical data handling pipeline improves performance of qPCR-based circulating miRNA measurements. RNA, 23, 811-821. PMID: 28202710. doi: 10.1261/rna.059063.116.

Ruiz-Villalba, A. et al. (2021) Use and Misuse of Cq in qPCR Data Analysis and Reporting. Life, 11, 496. PMID 34072308. doi: 10.3390/life11060496.