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Eppendorf Reference® 2

User Adjustment

Trademarks

Reference 2 - Adjustment User - en-01/052013

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1 User adjustment

The systematic error for Reference 2 pipettes can be changed via the user adjustment or factory adjustment.

This document provides information about when you should change the user adjustment and what needs to be considered in the process. User adjustment is especially useful if the Research plus Reference 2 adjustment is only to be changed for a limited period. The adjustment can be reset to its original state at any time using the adjustment tool. The *Factory adjustment* document on the Reference 2 CD contains a description on how to change the factory adjustment of a Reference 2.

Make sure you read the general notes.

A change to the user adjustment is shown in the adjustment display.

1.1 Adjusting pipettes

NOTICE! Incorrect dispensing volume due to differences in temperature and for special liquids.
Temperature differences between the pipette, pipette tips and liquid; or solutions with physical data that is very different from that of water; may result in incorrect dispensing volumes.

- Avoid temperature differences the between pipette, pipette tip and liquid.
- Ensure that the temperature is between 20 °C and 27 °C and remains constant at ±0.5 °C.
- Check the dispensing volume and make sure that you can affirm all the questions listed in the general information.

The random and systematic errors recorded on delivery can be found in the *Eppendorf Certificate*. This certificate is included in delivery. Changes to the factory adjustment will render the certificate void.

1.1.1 General instructions on user and factory adjustment

The Reference 2 was adjusted and tested prior to delivery. The adjustment opening is sealed using a gray plastic adjustment seal. The adjustment seal is labeled with the abbreviation "ADJ". The adjustment display shows "0".

Changing the adjustment of the Reference 2 is sometimes recommended for solutions that are very different from water in terms of their density, viscosity, surface tension and/or vapor pressure, etc. If the density of an aqueous solution changes by approximately ±10%, for example, due to the salt concentration, the volume changes by approximately ±0.2%. This statement does not apply if other relevant properties of the liquid also change.

If the pipette is used at extremely high altitudes, it must be calibrated to the ambient air pressure. At 1 000 m above sea level, the volume error of a 100 µL pipette is about –0.3%.

When using special tips, i.e. tips with a geometry that is very different from standard tips, changing the adjustment can improve the dispensing accuracy (systematic error).

Follow the adjustment recommendations for epT.I.P.S. long.

Adjustment changes can be reset using simple steps.

In addition to changing the user adjustment, a Reference 2 can be permanently changed by altering the factory adjustment.

Changes to the user or factory adjustment do not affect the dispensing precision (random error). Precision can be improved by exchanging worn parts. Precision is also significantly affected by handling.

The existing dispensing volume must be checked before changing the factory calibration or adjustment.

The actual volume can be checked by weighing:

$$\text{Actual volume} = \frac{\text{Mean value of the weighings}}{\text{Density liquids at weighing temperature}}$$

The density of distilled water is approx. 0.9982 mg/µL at 20°C and 0.9965 mg/µL at 27°C.

If the set volume is the same as the actual volume, no correction is necessary.

If there is a difference between the actual volume and the set volume of distilled water, please check the following:

- Is there any liquid dripping from the tip?
- Is the pipette tip fitted leak-proof?
- Is the tip cone undamaged?
- Are the piston and cylinder leak-proof?
  Adequate leak tightness is ensured when no drop forms on the pipette tip after aspiration of the nominal volume with distilled water and a waiting time of approx. 15 s. Hold the pipette vertically without touching the pipette tip. Prewet the tip several times for nominal volumes ≤ 20 µL.
- Does the temperature of the pipetted liquid correspond to:
  - The temperature of the device?
  - The ambient air temperature?
- Is the weighing location free of drafts?
- Does the work method and pipetting speed permit complete aspiration and dispensing of the liquid?
- Has the correct numerical value for "Density liquids at weighing temperature" been used to calculate the actual volume?
- Is the set volume correct?
- For very small volumes (<10 µL): Is the fine balance sufficiently sensitive (balance resolution: 0.001 mg)?
- Were original epT.I.P.S pipette tips used for testing?

No adjustment changes are allowed unless you can answer all the questions with "yes". In all other cases, the problems associated with the questions answered with "no" must be eliminated. If the problem is remedied by exchanging a complete lower part or other parts that affect the volume, proper assembly must be verified by carrying out a gravimetric test. Information on the systematic and random errors to be met can be found in the "Technical data" chapter.

1.1.2 Changing the user adjustment

If the adjustment is changed, the volume changes by a certain value. Strictly speaking, the change only applies to the testing volume.

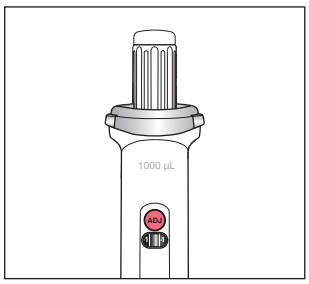
Example:

You readjust a 10 – 100 µL pipette with a volume setting of 100 µL by 1 µL (1 µL ≙ 1%). If the volume setting is 10 µL, the pipette is also adjusted by 1 µL (≙ 10%).

Auxiliary aids from the delivery package

- Pin
- Red plastic adjustment seal (ADJ)

1. Insert the pin in the middle of the gray adjustment seal (ADJ).
2. Remove the adjustment seal.
3. Insert the adjustment tool.
4. Turn the adjustment tool until the adjustment display shows the desired value.
5. Read the set value distortion-free using the aligning aid in the viewing window.
6. Carry out weighings to check the accuracy and precision.



7. Attach the red adjustment seal after the inspections.

1.2 Volume change obtained by modifying the adjustment display

Tab. 1-1: Single-channel Reference 2

Nominal volume, color code	**+8 Δ V**	**+6 Δ V**	**+4 Δ V**	**+2 Δ V**	**-2 Δ V**	**-4 Δ V**	**-6 Δ V**	**-8 Δ V**
1 µL, 2 µL, 2,5 µL, dark gray	0.05 µL	0.0375 µL	0.025 µL	0.0125 µL	-0.0125 µL	-0.025 µL	-0.0375 µL	-0.05 µL
5 µL,10 µL, medium gray	0.2 µL	0.15 µL	0.1 µL	0.05 µL	-0.05 µL	-0.1 µL	-0.15 µL	-0.2 µL
20 µL, light gray	0.4 µL	0.3 µL	0.2 µL	0.1 µL	-0.1 µL	-0.2 µL	-0.3 µL	-0.4 µL
10 µL, 20 µL, yellow	0.4 µL	0.3 µL	0.2 µL	0.1 µL	-0.1 µL	-0.2 µL	-0.3 µL	-0.4 µL
25 µL, 50 µL, 100 µL, yellow	2 µL	1.5 µL	1 µL	0.5 µL	-0.5 µL	-1 µL	-1.5 µL	-2 µL
200 µL, yellow	4 µL	3 µL	2 µL	1 µL	-1 µL	-2 µL	-3 µL	-4 µL
300 µL, orange	6 µL	4.5 µL	3 µL	1.5 µL	-1.5 µL	-3 µL	-4.5 µL	-6 µL
200 µL, 250 µL, 500 µL, 1000 µL, blue	20 µL	15 µL	10 µL	5 µL	-5 µL	-10 µL	-15 µL	-20 µL
2 mL, 2,5 mL, red	0.05 mL	0.0375 mL	0.025 mL	0.0125 mL	-0.0125 mL	-0.025 mL	-0.0375 mL	-0.05 mL
5 mL, purple	0.1 mL	0.075 mL	0.05 mL	0.025 mL	-0.025 mL	-0.05 mL	-0.075 mL	-0.1 mL
10 mL, turquoise	0.2 mL	0.15 mL	0.1 mL	0.05 mL	-0.05 mL	-0.1 mL	-0.15 mL	-0.2 mL

Tab. 1-2: Reference 2 multi-channel

Nominal volume, color code	**+8 Δ V**	**+6 Δ V**	**+4 Δ V**	**+2 Δ V**	**-2 Δ V**	**-4 Δ V**	**-6 Δ V**	**-8 Δ V**
10 µL, medium gray	0.2 µL	0.15 µL	0.1 µL	0.05 µL	-0.05 µL	-0.1 µL	-0.15 µL	-0.2 µL
100 µL, yellow	2 µL	1.5 µL	1 µL	0.5 µL	-0.5 µL	-1 µL	-1.5 µL	-2 µL
300 µL, orange	6 µL	4.5 µL	3 µL	1.5 µL	-1.5 µL	-3 µL	-4.5 µL	-6 µL

Explanation: The Δ volume values (Δ V) listed above are theoretical values and only serve as examples. They apply to every set volume in pipettes with adjustable volume settings. Depending on the mode of operation and other conditions (temperature, density, etc.), the actual values may differ from the above values. This applies to all pipettes. Each change to an adjustment must be gravimetrically tested.

1.3 Reference 2 settings for 50 % glycerine

Tab. 1-3: Single-channel Reference 2

Nominal volume, color code	Nominal volume, adjustment display in position	50 % of the nominal volume, adjustment display in position
2.5 µL, dark gray	No setting change required	No setting change required
10 µL, medium gray	No setting change required	No setting change required
20 µL, light gray	+1 (~ +0.05 µL)	No setting change required
20 µL, yellow	+1 (~ +0.05 µL)	No setting change required
100 µL, yellow	+1 (~ +0.25 µL)	+1 (~ +0.25 µL)
200 µL, yellow	+1 (~ +0.5 µL)	+1 (~ +0.5 µL)
300 µL, orange	+1 (~ +0.75 µL)	+1 (~ +0.75 µL)
1000 µL, blue	+1 (~ +2.5 µL)	+1 (~ +2.5 µL)
2.5 mL, red	+1 (~ +0.006 mL)	+1 (~ +0.006 mL)
5 mL, purple	+1 (~ +0.013 mL)	+0.5 (~ +0.0063 mL)
10 mL, turquoise	+2 (~ +0.05 mL)	+0.5 (~ +0.013 mL)

Tab. 1-4: Reference 2 multi-channel

Nominal volume, color code	Nominal volume, adjustment display in position	50 % of the nominal volume, adjustment display in position
10 µL, medium gray	No setting change required	No setting change required
100 µL, yellow	No setting change required	No setting change required
300 µL, orange	+0.5 (~ +0.38 µL)	+0.5 (~ +0.38 µL)

Explanation: The above settings are only informative because systematic and random errors are influenced by handling, the tip used, and other issues (e.g., the temperature). The above settings were determined for a 50 % (w/w) aqueous glycerol solution at room temperature. In the cells, the stroke volume change is indicated in parentheses after the setting for the adjustment display. The glycerol solution used had a density of 1.1238 g/mL (= mg/µL) at 25 °C. The data was calculated for wall dispensing. The blow-out was triggered approx. 3 seconds after dispensing. The tips were not prewetted. A new tip was used for each dispensing operation. The work was carried out relatively quickly and, therefore, under realistic conditions. It is imperative that the data be checked according to your own work method.

Technical specifications subject to change.

1.4 Reference 2 setting values for 45 % cesium chloride

Tab. 1-5: Single-channel Reference 2

Nominal volume, color code	Nominal volume, adjustment display in position	50 % of the nominal volume, adjustment display in position
2.5 µL, dark gray	No data determined	No data determined
10 µL, medium gray	+6.5 (~ +0.15 µL)	+3.5 (~ +0.09 µL)
20 µL, light gray	+6.5 (~ +0.33 µL)	+2.5 (~ +0.13 µL)
20 µL, yellow	+6.5 (~ +0.33 µL)	+2.5 (~ +0.13 µL)
100 µL, yellow	+3 (~ +0.75 µL)	+3 (~ +0.75 µL)
200 µL, yellow	+2.5 (~ +1.25 µL)	+2 (~ +1 µL)
300 µL, orange	+2.5 (~ +1.9 µL)	+2.5 (~ +1.9 µL)
1000 µL, blue	+2 (~ +5 µL)	+2 (~ +5 µL)
2.5 mL, red	+1.5 (~ +0,01 mL)	+1.5 (~ +0,01 mL)
5 mL, purple	+1.5 (~ +0.02 mL)	+1.5 (~ +0.02 mL)
10 mL, turquoise	+5 (~ +0.13 mL)	+4 (~ +0.1 mL)

Tab. 1-6: Reference 2 multi-channel

Nominal volume, color code	Nominal volume, adjustment display in position	50 % of the nominal volume, adjustment display in position
10 µL, medium gray	+3 (~ +0.08 µL)	+3 (~ +0.08 µL)
100 µL, yellow	+1 (~ +0.025 µL)	+1 (~ +0.25 µL)
300 µL, orange	+1 (~ +0.75 µL)	+1 (~ +0.75 µL)

Explanation: The above settings are only informative because systematic and random errors are influenced by handling, the tip used, and other issues (e.g., the temperature). The settings listed above were determined for a 45 % (w/v) aqueous cesium chloride solution at room temperature. In the cells, the stroke volume change is indicated in parentheses after the setting for the adjustment display. The cesium chloride solution used has a density of 1.5010 g/mL (= mg/µL) at 22 °C. The data was calculated for wall dispensing. The blow-out was triggered approx. 3 seconds after dispensing. The tips were not prewetted. A new tip was used for each dispensing operation. The work was carried out relatively quickly and, therefore, under realistic conditions. It is imperative that the data be checked according to your own work method.

Technical specifications subject to change.

1.5 Setting for the epT.I.P.S. 1 250 µL L and 10 mL L

The piston stroke in each Reference 2 is optimized for the tip geometry of the corresponding epT.I.P.S. Using other tips may cause differences in the systematic errors (accuracy). When using epT.I.P.S. with dark gray, medium gray and light gray trays with the matching gray Reference 2, the differences among the systematic errors are so low that no correction is absolutely necessary. The same concept applies when using tips with orange trays and the yellow Reference 2. It also applies to tips with the tray color yellow and the Research plus with the color code orange.

Information on which tip is suitable for which pipette, and whether the tip limits volume absorption, is provided in the tables in the document *Ordering information* on the Reference 2 CD.

Very long tips or tips with a different shape can result in volume errors due to the filling height in the tip and the resulting air cushion between the liquid and the piston. These errors can be minimized by changing the user adjustment if very high demands are placed on systematic errors.

With the following combinations, the user adjustment can be changed to minimize the systematic error:

- epT.I.P.S. 1250 µL L (length 103 mm, dark green) and Reference 21000 µL, blue
- epT.I.P.S. 10 mL (length 243 mm, turquoise) and Reference 2 10 mL, turquoise

The following two tables show the deviations and settings for the user adjustment for the Research plus under the following conditions:

- Use of demineralized water
- Pipetting at room temperature
- Tip prewetted
- Tip immersion depth approx. 5 mm
- Slow aspiration and dispensing of water
- Blow-out triggered approx. 2 seconds with a time delay
- Vertical aspiration if possible, and wall dispensing at a slight angle

Tab. 1-7: Setting of the Reference 2with epT.I.P.S. 1 250 µL L and 10 mL L

Tip and pipette	Volume setting	Approximate deviation	Recommended user adjustment setting	Theoretical volume correction, valid for the entire measuring range
epT.I.P.S. 1 250 µL L 103 mm, dark green **Research plus** 1000 µL, blue	1 000 µL 500 µL	-10 µL -9 µL	+4 +4	+10 µL +10 µL
epT.I.P.S. 10 mL L 243 mm, turquoise **Research plus** 10 mL, turquoise	10 mL 5 mL	-0.13 mL -0.05 mL	+5 +2	+0.125 mL +0.05 mL

For 10 mL epT.I.P.S., it is advisable to change the adjustment to the respective volume range used. For single dispensing operations, you should also consider a correspondingly increased volume setting as an alternative.

Since the measured values depend greatly on your personal working method, carry out your own gravimetric tests to check all recommended settings for the user adjustment.

1.6 Settings for additional liquids

Tab. 1-8: Single-channel Reference 2, Setting for pipetting the nominal volume

Nominal volume, color code	Caustic soda NaOH 40 % Density: 1.437 mg/µL	Phosphoric acid H_3PO_4 85 % Density: 1.689 mg/µL	Dimethyl sulfoxide DMSO 99.8 % Density: 1.097 mg/µL
20 µL, light gray	+3 (~ +0.15 µL)	0	-4 (~ -0.2 µL)
20 µL, yellow	+5 (~ +0.25 µL)	0	-4 (~ -0.2 µL)
100 µL, yellow	0	+1 (~ +0.25 µL)	-4 (~ -1 µL)
200 µL, yellow	+2 (~ +1 µL)	+2 (~ +1 µL)	-4 (~ -2 µL)
300 µL, orange	+3 (~ +2.25 µL)	+2 (~ +1.5 µL)	-4 (~ -3 µL)
1 000 µL, blue	+0.5 (~ +1.25 µL)	+2 (~ +5 µL)	-2 (~ -5 µL)
2.5 mL	+2 (~ +0.012 mL)	+3 (~ +0.015 mL)	-1 (~ -0.006 mL)
5 mL, purple	+4 (~ +0.05 mL)	+5 (~ +0.063 mL)	-2 (~ -0.025 mL)
10 mL, turquoise	+6 (~ +0.15 mL)	+8 (~ +0.2 mL)	0

Explanation: The above settings are only informative because systematic and random errors are influenced by handling, the tip used and other issues (e.g., the temperature). In the cells the respective stroke volume change of the Reference 2 is indicated in brackets behind the setting for the adjustment display. The data was calculated for wall dispensing. The blow-out was triggered approx. 3 seconds after dispensing. The tips were not prewetted. A new tip was used for each dispensing operation. The work was carried out relatively quickly and, therefore, under realistic conditions. It is imperative that the data be checked according to your own work method.The deviating behavior of dimethyl sulfoxide (DMSO) can be explained by the capillary action when the tip is dipped into the liquid.

Technical specifications subject to change.

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