

The Eppendorf Mechanical Pipettes Research® plus and Reference 2 – Fully Autoclavable, Easy Adjustment, Quick and Simple Maintenance

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Abstract

Research plus and Reference 2 pipettes are easy to clean and maintain. Hence, routine maintenance can be easily performed by the user, saving valuable time. Readjustment of the pipette for liquids whose physical properties differ significantly from those of water is also easy and

safe to perform. This Application Note demonstrates the special features of maintaining and cleaning Research plus pipettes and provides detailed information on changing the pipette's adjustment.

Introduction

Research plus and Reference 2 are manual pipettes with different features and functional principles (e.g. one-button / two-button operation). Fulfilling the highest requirements, both pipettes have equal functionalities in terms of decontamination, maintenance and adjustment. In order to meet the high requirements of a modern laboratory, high quality piston stroke pipettes should be partly or fully autoclavable as well as UV resistant.

The Eppendorf Research plus and Reference 2 pipettes can be decontaminated either by UV light or by autoclaving the entire instrument. Thus, cleaning following use with infectious or contaminated samples is easily possible.

Adjustment for specific liquids or altitude

During production, piston stroke pipettes are adjusted to distilled water under certified measuring conditions. To indicate the adjustment, all Research plus pipettes carry an adjustment seal (Fig. 1). If necessary, adjustment for specific liquids or for altitude can be carried out easily by the user. The red adjustment seal, which is applied to the adjustment opening following an adjustment, serves to visualize a change of adjustment settings (Fig. 1).

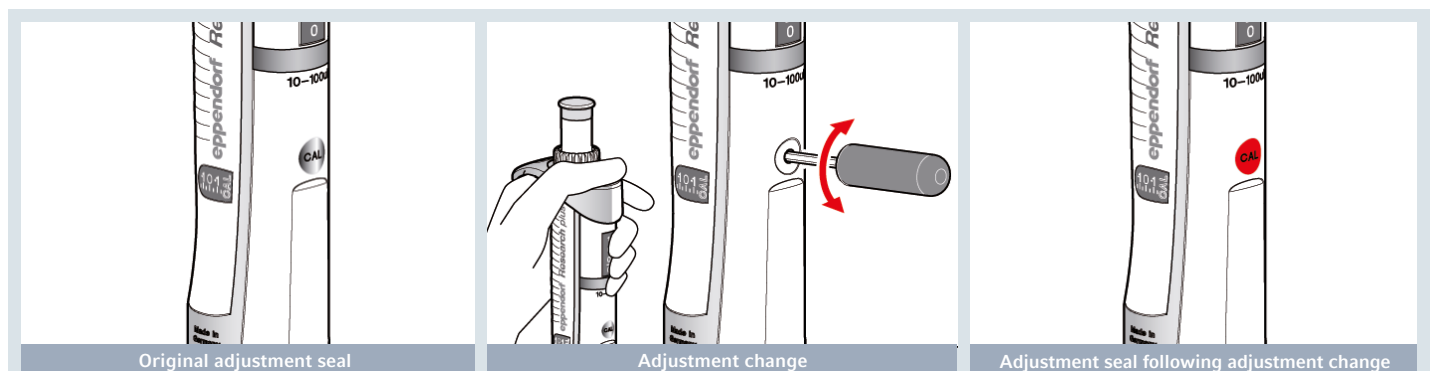
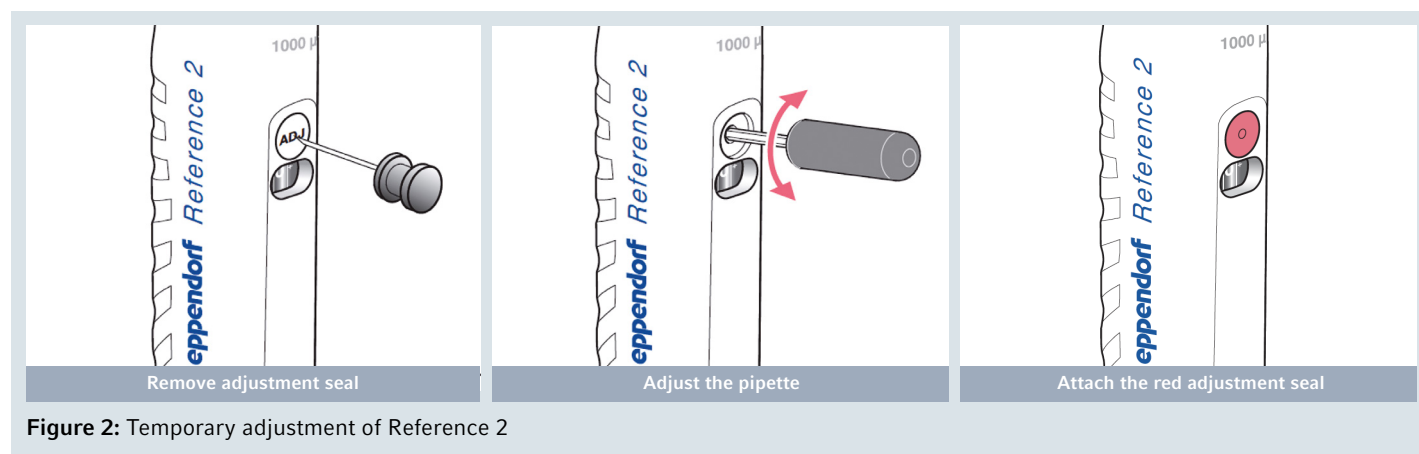
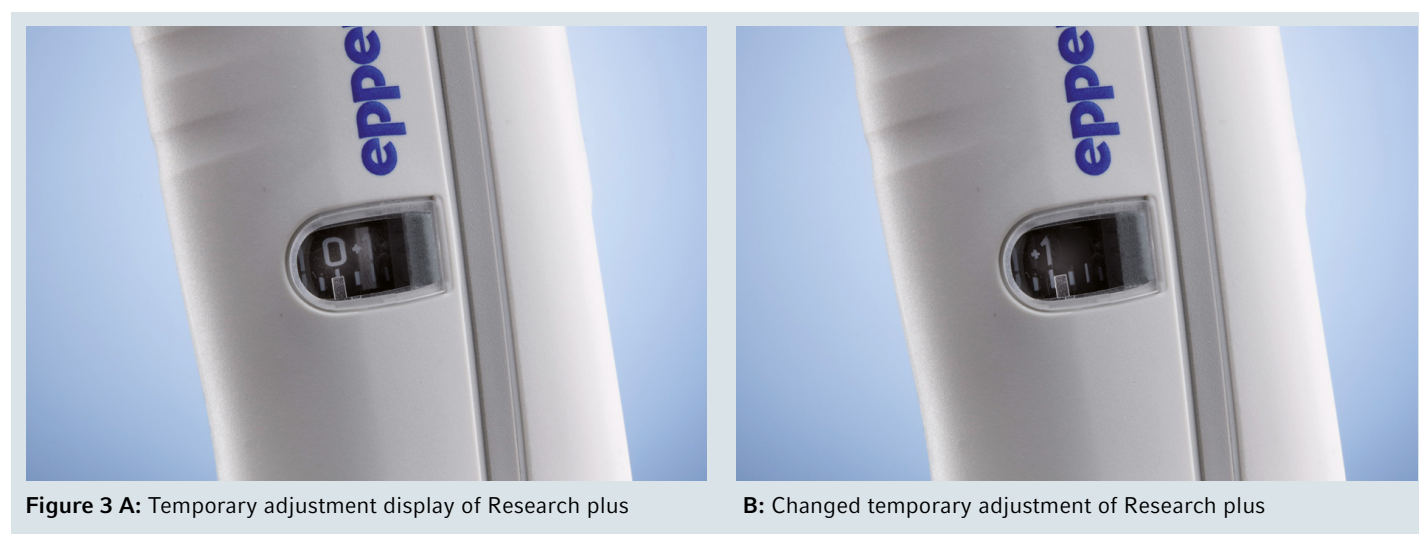


Figure 1: Temporary adjustment of Research plus



An innovative feature of the Research plus and Reference 2 pipettes is the additional indication of a change to the temporary adjustment through the adjustment window (Fig. 3). Here, the exact change of adjustment is immediately visible (Fig. 3). Using the adjustment window, changing of the

adjustment back to the original setting is easily performed. An easy-to-use adjustment option is especially interesting for the work with liquids which density differs from water or if elongated tips are to be used. In every case, a change in adjustment needs to be verified gravimetrically.



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

Example: You adjust 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%).

1. Remove the grey calibration seal.
2. Keep the ejector pressed.
3. Insert the adjustment tool (from the delivery package).
4. Turn the adjustment tool until the desired value is displayed on the adjustment display.
5. Carry out weighings to verify accuracy and precision.
6. After the checks, stick the red adjustment seal (from the delivery package) on.

If the adjustment is meant for a specific liquid, mark the pipette accordingly. Use the labeling area on the pipette for this purpose and write down the liquid and the volume. Carry out a gravimetric test for each change to the adjustment. Follow the test procedures of EN ISO 8655-2 and 8655-6. An SOP (Standard Operation Procedure) for the test is available on our website www.eppendorf.com. Here, further information about the temporary adjustment is also available.

Setting values Research plus and Reference 2

The settings listed in tab. 1 and tab. 2 are only informative because systematic and random errors are influenced by handling, the tip used and other issues (e.g. the temperature). The settings were determined for displayed concentrations at room temperature. 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 are subject to change. To change the factory adjustment please refer to the adjustment manual (www.eppendorf.com).

Table 1: Temporary adjustment settings for Research plus and Reference 2 single channel at nominal volume, different chemicals

Pipette	Chemicals						
	Glycerol 50%	Cesium chloride (CsCl) 45%	Caustic soda (NaOH) 40%	Phosphoric acid (H ₃ PO ₄) 85%	Sulfuric acid (H ₂ SO ₄) 96%	Dimethyl sulfoxide (DMSO) 99.8%	Polyethylen-glycol (PEG) 400 40%
0.1–2.5 µL ■ dark gray	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.5–10 µL ■ medium gray	0	6.5	n.d.	n.d.	n.d.	n.d.	n.d.
2–20 µL ■ light gray	1	6.5	3	0	0	-4	0
2–20 µL ■ yellow	1	6.5	5	0	0	-4	0
10–100 µL ■ yellow	1	3	0	1	0	-4	0
20–200 µL ■ yellow	1	2.5	2	2	0	-4	0
30–300 µL ■ orange	1	2.5	3	2	0	-4	0
100–1000 µL ■ blue	1	2	0.5	2	2	-2	0
0.25–2.5 mL ■ red	1	1.5	2	3	n.d.	-1	n.d.
0.5–5 mL ■ violet	1	1.5	4	5	5	-2	0
1–10 mL ■ turquoise	2	5	6	8	8	0	0

Table 2: Temporary adjustment settings for 8- and 12-channel Research plus and Reference 2 at nominal volume, different chemicals

Pipette	Chemicals	
	Glycerol 50%	Cesium chloride (CsCl) 45%
0.5–10 µL ■ medium gray	0	3
10–100 µL ■ yellow	0	1
30–300 µL ■ orange	0.5	1

Very long tips with 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 temporary adjustment if very high demands are placed on systematic errors. The following table (tab. 3) shows the deviations and settings for the temporary adjustment for the Research plus and Reference 2 under 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 with a time delay of approx. 2 seconds
- > vertical aspiration if possible, and wall dispensing at a slight angle

Table 3: Temporary adjustment settings for Research plus and Reference 2 single channel at nominal volume, elongated tips, water

Pipette Tip	epT.I.P.S.® 1250 µL L, 103 m ■ dark green		epT.I.P.S.® 10 mL L, 243 mm, ■ turquoise	
Pipette	100-1,000 µL		1-10 mL	
Set volume	1000 µL	500 µL	10 mL	5 mL
Temporary adjustment setting	4	4	5	2
Approximate deviation	-10 µL	-9 µL	-0.13 mL	-0.05 mL
Theoretic volume correction, valid for the entire measuring range	10 µL	10 µL	0.125 mL	0.05 mL

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. Therefore see tab. 3. Since the measured values depend greatly on your personal working method, carry out your own gravimetric tests to check all recommended settings for the temporary adjustment.

Material of piston and cylinder

In order to reduce the handling forces as well as the weight of the Research plus and Reference 2 pipettes, high quality synthetic materials were used in the production of pipette pistons of the 20 µL (yellow) to 10 mL volume range. The material PPS (polyphenylene sulfide, Fortron®) was used for the cylinder, the piston rod is made from PEI (polyetherimide), and the washer is made from EPDM (ethylene-propylene-diene-rubber). These tried and tested materials were subjected to chemical resistance testing over an extended period of time (100,000 strokes) using different substances, such as ethanol, acetone, concentrated hydrochloric acid and nitric acid. During the test phase, all plastics demonstrated very good chemical resistance. Furthermore, they showed high temperature resistance. More information about the resistance against chemicals and decontaminating reagents is given on the eppendorf website www.eppendorf.com. The pistons of pipettes of the volume range 0.1–20 µL (gray) are made from corrosion-free vanadium steel.

Reliability test

In the context of the reliability test, the following tests listed below were performed with the Research plus and Reference 2 pipettes:

- > 100,000 x tip attachment
- > 100,000 x tip ejection strokes
- > 200,000 x strokes including excess stroke
- > 50,000 x volume adjustment
- > 250 x pulling off of the ejection case
- > 200 x detachment and re-attachment of the lower part
- > 175 autoclave cycles (121 °C, 1 bar, 20 min.)

The number of respective tests chosen corresponds to an average 5 year use of the pipette. Additional tests included change of the adjustment, falling tests and gravimetric tests according to EN ISO 8655. The results showed that Eppendorfs pipette families Research plus and Reference 2 fully meet the testing requirements and did not show any signs of damage or impairment following the testing.

Both, the carefully chosen materials as well as the pipettes reliability offer broad cleaning and decontamination possibilities. They make the maintenance considerably easy, as shown in the following chapters.

Autoclaving and UV-sterilization

The piston stroke pipettes used today shall be either fully autoclavable, or the parts which become contaminated during improper use can be autoclaved. Autoclaving of air cushion pipettes and pipette tips (with the exception of filter tips) is normally performed at 121 °C at an excess pressure of 1 bar (100 kPa) for 20 min.

The Eppendorf pipettes Research plus and Reference 2 can be fully autoclaved. Following the autoclave cycle, the pipette needs to dry completely and cool down. In case the pipette was autoclaved in parts, all parts need to cool completely prior to re-assembly. Otherwise, plastic parts may be overexpanded and damaged. In the praxis most often several pipettes are processed in terms of maintenance and decontamination. Here it is important to ensure that upper and lower parts of one pipette become reassembled after e.g. decontamination. Avoiding the mixing of parts the Reference 2 has its serial number printed on the upper and the lower part. Greasing of the pipette piston-cylinder-system following autoclaving is not necessary with Eppendorf pipettes.

The UV resistance of the plastic materials used in a pipette is of central importance for many areas of application. UV-resistant pipettes, such as the Research plus and Reference 2 pipettes, can remain in areas of cell culture labs without risk, since the UV light used to disinfect these work areas will not have any adverse effects on the pipette material, nor on the function of the pipette.

During decontamination using UV light, the following parameters should be considered: A 30 Watt low-pressure mercury-vapor lamp with a characteristic wavelength of 254 nm is to be used. The optimal distance between lamp and pipette is approximately 60 cm.

The following methods for cleaning or decontamination of the Research plus pipette may not be combined:

- > Decontaminating reagents like disinfectants or DNA/RNA-decontaminants with additional
- > Steam autoclaving or UV-irradiation.

If you wish to apply a chemical decontamination prior to an UV irradiation or autoclaving always make sure that the decontaminating reagent has been wiped or rinsed off the pipette's surface with demineralized water.

Specifics regarding maintenance and cleaning

The Research plus and Reference 2 pipettes are easy to clean. The lower part should be cleaned on a regular basis, in accordance with the frequency of use (Fig. 4).

To this end, the ejector sleeve (4.1) will be pulled off while the ejection button is pushed down. The ring on the lower part with the label "PUSH TO RELEASE" (4.2) has to be slid up by approximately 5 mm (4.3) until the lower part is released. Subsequently, the lower part is to be taken out of the upper part (4.4).

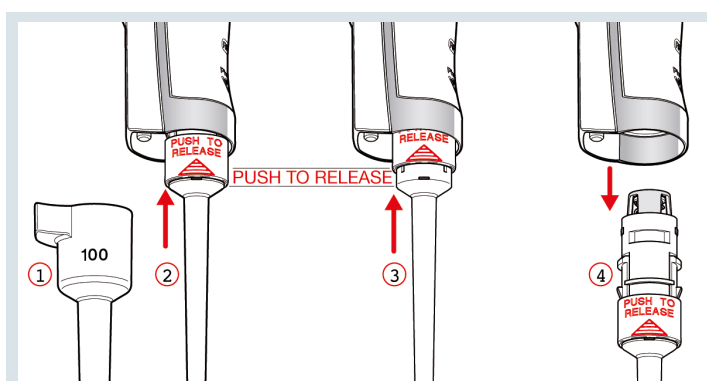


Figure 4: Removing the lower part

Specifics regarding maintenance and cleaning

For the multichannel pipettes, opening of the lower part occurs as follows: The lever (5.1) on the lower part has to be slid to the left or right (5.2). The lower part is released and can be removed. Put down the lower part with the lever facing downwards and slide the two latches down (5.3). For re-assembly, push the lower part into the upper part until it engages audibly.

The individual pistons of the multichannel pipettes can be released from their holders for cleaning. For this purpose, position a pipette tip behind the piston (6.1) and take the piston off the upper rail (6.2). Subsequently, pull the piston carefully out in an upward direction.

Take the tip cone at the lower end and push slightly upwards (6.3) and take it off the lower rail (6.4). Then remove the tip cone, including the cylinder and the spring, from their mounting (6.5). Re-assembly is performed in reverse order.

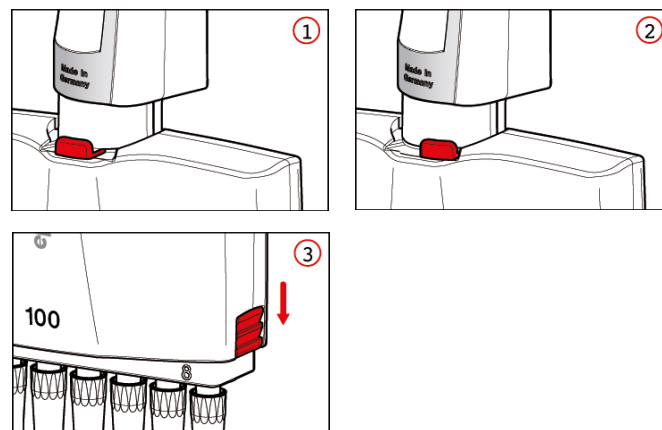


Figure 5: Opening of the multichannel lower part

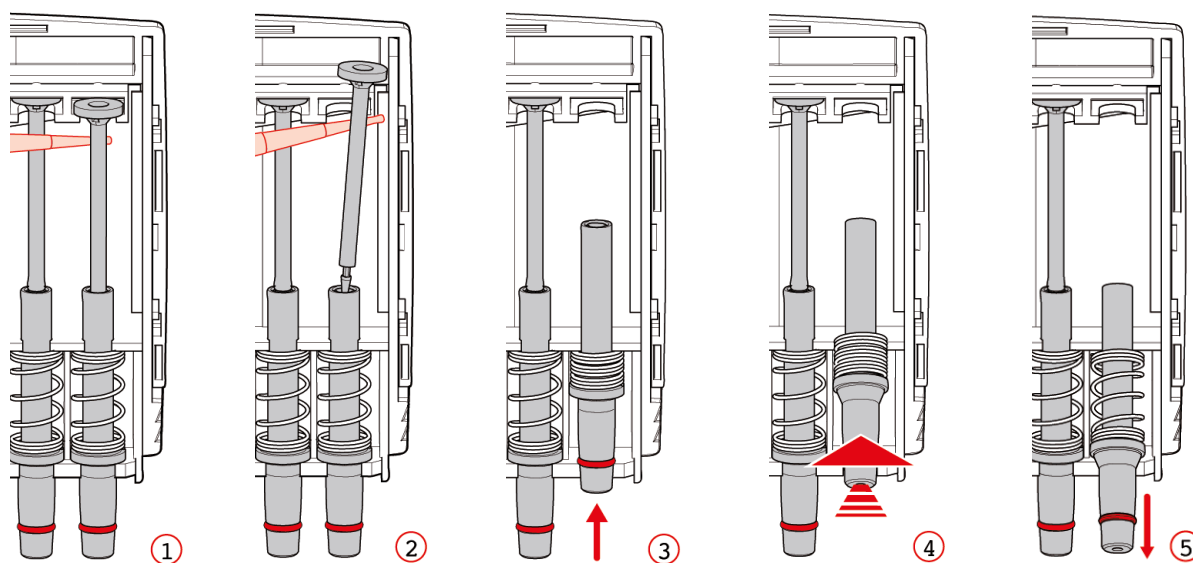


Figure 6: Removal and installing channels with multi-channel pipettes

Conclusion

In today's environment, function and handling forces of a pipette play important roles; however, additional deciding factors include reliability, simple cleaning and maintenance procedures, as well as decontamination by autoclaving and UV-irradiation. The simple and secure adjustment of

a piston stroke pipette is one further demand during daily laboratory routine. The Research plus and Reference 2 pipettes meet these criteria in every respect and are thus the ideal instrument for everyday pipetting in the laboratory.

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