

Register your instrument!  
[www.eppendorf.com/myeppendorf](http://www.eppendorf.com/myeppendorf)



# Eppendorf Reference<sup>®</sup> 2

**Adjustment**

Copyright© 2015 Eppendorf AG, Germany. All rights reserved, including graphics and images. No part of this publication may be reproduced without the prior permission of the copyright owner.

Eppendorf® and the Eppendorf logo are registered trademarks of Eppendorf AG, Germany.

Combitips®, epT.I.P.S.®, and Reference® 2 are registered trademarks of Eppendorf AG, Germany.

Registered trademarks and protected trademarks are not marked in all cases with ® or ™ in this manual.

Protected by U.S. Patent Nos. 7,434,484; 7,673,532; 7,674,432; 8,297,134

## Table of contents

<b>1 Prerequisites for making an adjustment. . . . .</b>	<b>5</b>
1.1 Factory adjustment . . . . .	6
1.2 User adjustment . . . . .	7
1.2.1 Adjustment example . . . . .	8
1.3 Change in volume resulting from a change in the user adjustment. . . . .	9
1.3.1 Change in volume for single-channel pipettes . . . . .	9
1.3.2 Change in volume for multi-channel pipettes. . . . .	9
1.4 Setting for epT.I.P.S. Long . . . . .	10
<b>2 Liquids with increased or reduced density. . . . .</b>	<b>11</b>
2.1 Caesium chloride CsCl . . . . .	11
2.1.1 Adjustment values for single-channel pipette. . . . .	11
2.1.2 Adjustment values for multi-channel pipette . . . . .	11
2.2 Glycerol C <sub>3</sub> H <sub>8</sub> O <sub>3</sub> . . . . .	12
2.2.1 Adjustment values for single-channel pipette. . . . .	12
2.2.2 Adjustment values for multi-channel pipette . . . . .	12
2.3 Caustic soda NaOH . . . . .	13
2.3.1 Adjustment values for single-channel pipette. . . . .	13
2.4 Phosphoric acid H <sub>3</sub> PO <sub>4</sub> . . . . .	14
2.4.1 Adjustment values for single-channel pipette. . . . .	14
<b>3 Capillary action during immersion of the pipette tip. . . . .</b>	<b>15</b>
3.1 Dimethyl sulfoxide DMSO. . . . .	15
3.1.1 Adjustment values for single-channel pipette. . . . .	15
<b>4 Measurement errors. . . . .</b>	<b>16</b>
4.1 Measurement errors according to Eppendorf. . . . .	16
4.1.1 Variable volume single-channel pipette . . . . .	16
4.1.2 Variable volume multi-channel pipette . . . . .	17
4.2 Maximum permissible errors in accordance with ISO 8655-2002 . . . . .	18

**Table of contents**

Eppendorf Reference® 2  
English (EN)

## 1 Prerequisites for making an adjustment

Making an adjustment sets the dispensing volume in such a way that systematic errors are minimized for the intended application. Making an adjustment changes the dispensing volume across the entire volume range by approximately the same volume.

A deviation between the actual volume and the set value can be due to different reasons. Before making an adjustment, check that the other reasons for a deviation in dispensing can be excluded.

- The tip cone is OK
- The pipette tip is compatible with the pipette
- The pipette is leak-tight
- Pre-wetting was sufficient
- The liquid, device and ambient air have the same temperature
- The method of working and pipetting speed are correct
- The balance resolution is adequate
- The weighing location is draft-free
- The calculation of the volume is correct

If it is recognized by means of gravimetric testing that a deviation that requires corrective action is present, the pipette must be adjusted.

Changing the adjustment is useful in the following cases:

- Liquids whose physical properties (density, viscosity, surface tension, vapor pressure) differ significantly from those of water
- Capillary action during the immersion of the pipette tip (e.g., in the case of DMSO)
- Changes in the atmospheric pressure due to the altitude at which the pipette is used
- Pipette tips whose geometry differs significantly from standard tips (e.g., ep T.I.P.S. long)

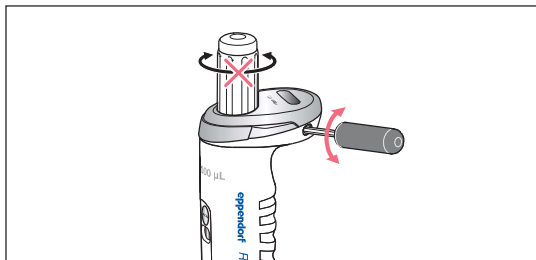
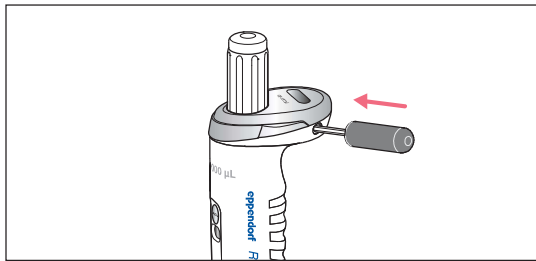
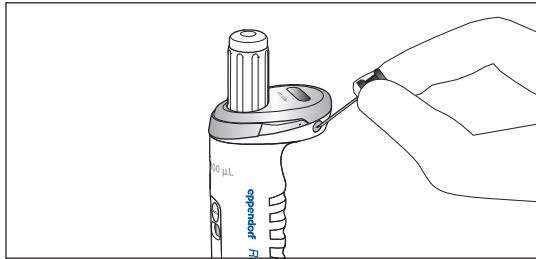


The change in the adjustment does not affect the random error of the dispensing procedure. The random error can be improved by exchanging worn parts. The random error is also strongly influenced by handling.

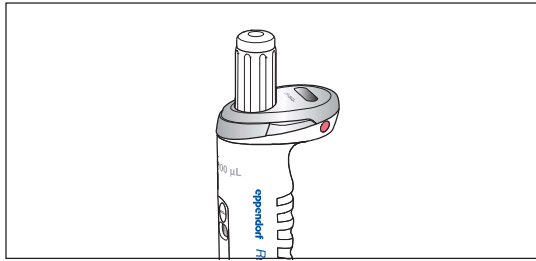
## 1.1 Factory adjustment

### Prerequisites

- Pin
- Red plastic safety plug
- Adjustment display set to "0" position



1. Check the device by carrying out a gravimetric test and note the results.
2. Use the pin to pierce the safety plug in the center.
3. Remove the safety plug.
4. Insert the adjustment tool.
5. Fix the control button.
6. Turn the adjustment tool to change the volume display.
7. Adjust the volumes that were gravimetrically determined during the test.
8. Check the setting of the testing volumes by carrying out a gravimetric test.



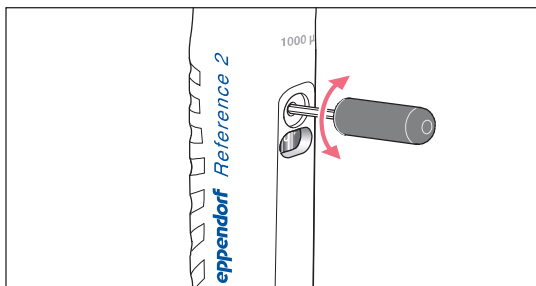
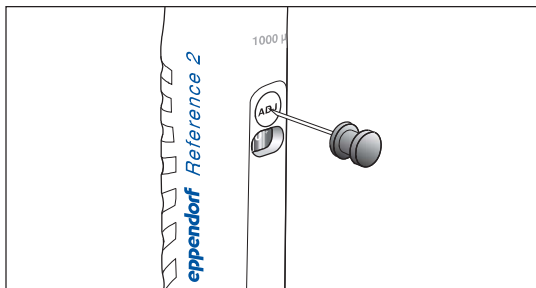
9. Insert the red safety plug.  
The red safety plug on the pipette indicates that the pipette has been adjusted and calibrated by the user.

## 1.2 User adjustment

A change in the user adjustment is clearly shown in the viewing window. The adjustment display is set to "0" on delivery. The factory setting can be restored by resetting to "0".

### Prerequisites

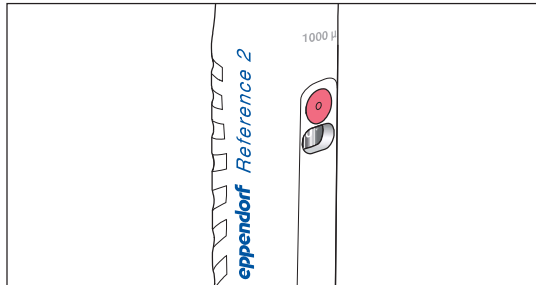
- Pin
- Red plastic adjustment seal



1. Determine the deviation between the dispensed volume and the set volume by carrying out a gravimetric test.
2. The value for the user adjustment results from the calculated deviation (see p. 9).
3. Use the pin to pierce the adjustment seal in the center.
4. Remove the adjustment seal.
5. Insert the adjustment tool.
6. Turn the adjustment tool until the adjustment display shows the desired value.

**Prerequisites for making an adjustment**

Eppendorf Reference® 2  
English (EN)



7. Check the setting of the testing volumes by carrying out a gravimetric test.

8. Insert the red adjustment seal.

9. Indicate the validity range of the pipette.  
The red adjustment seal on the pipette indicates that the pipette has been adjusted and calibrated by the user.

**1.2.1 Adjustment example****Problem**

- Set volume 300 µL
- Liquid dimethyl sulfoxide (DMSO)
- Due to capillary action, a volume of 303 µL is aspirated

**Solution**

- ▶ Set the user adjustment scale to -4.

The effective dispensing volume is reduced by approx. 3 µL to 300 µL.



### 1.3 Change in volume resulting from a change in the user adjustment

The volume values ( $\Delta V$ ) are theoretical values and provided for guidance purposes only. They apply to every set volume in pipettes with adjustable volume settings. Your method of working and other conditions (temperature, density etc.) may result in deviations to the values.

#### 1.3.1 Change in volume for single-channel pipettes

Nominal volume	Color code	User adjustment $\Delta V$ [ $\mu\text{L}$ ]							
		+8	+6	+4	+2	-2	-4	-6	-8
1 $\mu\text{L}$	dark gray	0,05	0,0375	0,025	0,0125	-0.0125	-0.025	-0.0375	-0.05
2 $\mu\text{L}$									
2.5 $\mu\text{L}$									
5 $\mu\text{L}$	medium gray	0.2	0.15	0.1	0.05	-0.05	-0.1	-0.15	-0.2
10 $\mu\text{L}$									
20 $\mu\text{L}$	light gray	0.4	0.3	0.2	0.1	-0.1	-0.2	-0.3	-0.4
10 $\mu\text{L}$	yellow	0.4	0.3	0.2	0.1	-0.1	-0.2	-0.3	-0.4
20 $\mu\text{L}$									
25 $\mu\text{L}$	yellow	2	1.5	1	0.5	-0.5	-1	-1.5	-2
50 $\mu\text{L}$									
100 $\mu\text{L}$									
200 $\mu\text{L}$	yellow	4	3	2	1	-1	-2	-3	-4
300 $\mu\text{L}$	orange	6	4.5	3	1.5	-1.5	-3	-4.5	-6
200 $\mu\text{L}$	blue	20	15	10	5	-5	-10	-15	-20
250 $\mu\text{L}$									
500 $\mu\text{L}$									
1000 $\mu\text{L}$									
2 mL	red	50	37.5	25	12.5	-12.5	-25	-37.5	-50
2.5 mL									
5 mL	violet	100	75	50	25	-25	-50	-75	-100
10 mL	turquoise	200	150	100	50	-50	-100	-150	-200

#### 1.3.2 Change in volume for multi-channel pipettes

Nominal volume	Color code	User adjustment $\Delta V$ [ $\mu\text{L}$ ]							
		+8	+6	+4	+2	-2	-4	-6	-8
10 $\mu\text{L}$	medium gray	0.2	0.15	0.1	0.05	-0.05	-0.1	-0.15	-0.2
100 $\mu\text{L}$	yellow	2	1.5	1	0.5	-0.5	-1	-1.5	-2
300 $\mu\text{L}$	orange	6	4.5	3	1.5	-1.5	-3	-4.5	-6

## 1.4 Setting for epT.I.P.S. Long

The geometry of the pipette tips differs significantly from standard tips. A smaller volume is aspirated when using epT.I.P.S. Long. It is therefore recommended to change the user adjustment to reduce the systematic error.

### Test conditions

- Use of demineralized water
- Pipetting at room temperature
- Tip pre-wetted
- 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

epT.I.P.S.	Pipette color code	Volume setting	Recommended user adjustment setting
1250 µL L	blue	500 µL	+4
		1000 µL	+4
5 mL L	violet	2.5 ml	+1,5
		5 mL	+1,5
10 mL L	turquoise	5 mL	+2
		10 mL	+5



The measured values depend heavily on your individual method of working. The recommended settings must be checked by means of gravimetric testing.

## 2 Liquids with increased or reduced density

It is recommended to adjust the pipette in the case of liquids with an increased or reduced density. The values for the adjustment are determined in the tables.



The measured values depend heavily on your individual method of working. The recommended settings must be checked by means of gravimetric testing.

### 2.1 Caesium chloride CsCl

#### Test conditions

- Concentration 45 %
- Temperature 22 °C
- Density 1.501 g/mL
- Liquid dispensing against the tube inner wall
- Blow-out 3 s after dispensing
- Pipette tip not pre-wetted
- A new pipette tip for each liquid dispensing operation

#### 2.1.1 Adjustment values for single-channel pipette

Nominal volume	Color code	Adjustment value 100% nominal volume	Adjustment value 50% nominal volume
2.5 µL	dark gray	–	–
10 µL	medium gray	+6,5	+3,5
20 µL	light gray	+6,5	+2,5
20 µL	yellow	+6,5	+2,5
100 µL	yellow	+3	+3
200 µL	yellow	+2,5	+2
300 µL	orange	+2,5	+2,5
1000 µL	blue	+2	+2
2.5 mL	red	+1,5	+1,5
5 mL	violet	+1,5	+1,5
10 mL	turquoise	+5	+4

#### 2.1.2 Adjustment values for multi-channel pipette

Nominal volume	Color code	Adjustment value 100% nominal volume	Adjustment value 50% nominal volume
10 µL	medium gray	+3	+3
100 µL	yellow	+1	+1
300 µL	orange	+1	+1

## 2.2 Glycerol C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>

### Test conditions

- Concentration 50%
- Temperature 25°C
- Density 1.124 g/mL
- Liquid dispensing against the tube inner wall
- Blow-out 3 s after dispensing
- Pipette tip not pre-wetted
- A new pipette tip for each liquid dispensing operation

### 2.2.1 Adjustment values for single-channel pipette

Nominal volume	Color code	Adjustment value 100% nominal volume	Adjustment value 50% nominal volume
2.5 µL	dark gray	0	0
10 µL	medium gray	0	0
20 µL	light gray	+1	0
20 µL	yellow	+1	0
100 µL	yellow	+1	+1
200 µL	yellow	+1	+1
300 µL	orange	+1	+1
1000 µL	blue	+1	+1
2.5 mL	red	+1	+1
5 mL	violet	+1	+0,5
10 mL	turquoise	+2	+0,5

### 2.2.2 Adjustment values for multi-channel pipette

Nominal volume, color code	Color code	Adjustment value 100% nominal volume	Adjustment value 50% nominal volume
10 µL	medium gray	0	0
100 µL	yellow	0	0
300 µL	orange	+0,5	+0,5

## 2.3 Caustic sod1wae77eea NaOH

### Test conditions

- Concentration 40%
- Temperature 25°C
- Density 1.437 g/mL
- Liquid dispensing against the tube inner wall
- Blow-out 3 s after dispensing
- Pipette tip not pre-wetted
- A new pipette tip for each liquid dispensing operation

### 2.3.1 Adjustment values for single-channel pipette

Nominal volume	Color code	Adjustment value 100% nominal volume
20 µL	light gray	+3
20 µL	yellow	+5
100 µL	yellow	0
200 µL	yellow	+2
300 µL	orange	+3
1000 µL	blue	+0,5
2,5 mL	red	+2
5 mL	violet	+4
10 mL	turquoise	+6

## 2.4 Phosphoric acid H<sub>3</sub>PO<sub>4</sub>

### Test conditions

- Concentration 85%
- Temperature 25°C
- Density 1.689 g/mL
- Liquid dispensing against the tube inner wall
- Blow-out 3 s after dispensing
- Pipette tip not pre-wetted
- A new pipette tip for each liquid dispensing operation

### 2.4.1 Adjustment values for single-channel pipette

Nominal volume	Color code	Adjustment value 100% nominal volume
20 µL	light gray	0
20 µL	yellow	0
100 µL	yellow	+1
200 µL	yellow	+2
300 µL	orange	+2
1000 µL	blue	+2
2,5 mL	red	+3
5 mL	violet	+5
10 mL	turquoise	+8

### 3 Capillary action during immersion of the pipette tip

Due to capillary action, the volume of liquid aspirated is larger in the case of liquids such as dimethyl sulfoxide. It is therefore recommended to change the user adjustment to reduce the systematic error.



The measured values depend heavily on your individual method of working. The recommended settings must be checked by means of gravimetric testing.

#### 3.1 Dimethyl sulfoxide DMSO

##### Test conditions

- Concentration 99.8%
- Temperature 25°C
- Density 1.097 g/mL
- Liquid dispensing against the tube inner wall
- Blow-out 3 s after dispensing
- Pipette tip not pre-wetted
- A new pipette tip for each liquid dispensing operation

##### 3.1.1 Adjustment values for single-channel pipette

Nominal volume	Color code	Adjustment value 100% nominal volume
20 µL	light gray	-4
20 µL	yellow	-4
100 µL	yellow	-4
200 µL	yellow	-4
300 µL	orange	-4
1000 µL	blue	-2
2,5 mL	red	-1
5 mL	violet	-2
10 mL	turquoise	0

## 4 Measurement errors

### 4.1 Measurement errors according to Eppendorf

#### 4.1.1 Variable volume single-channel pipette

Model	epT.I.P.S. test tip	Testing volume	Error			
			Systematic error		Random error	
			± %	± µL	± %	± µL
0.1 µL – 2.5 µL	0.1 µL – 10 µL dark gray 34 mm	0.1 µL	48,0	0,048	12,0	0,012
		0,25 µL	12,0	0,03	6,0	0,015
		1,25 µL	2,5	0,031	1,5	0,019
		2,5 µL	1,4	0,035	0,7	0,018
0.5 µL – 10 µL	0.1 µL – 20 µL medium gray 40 mm	0,5 µL	8,0	0,04	5,0	0,025
		1 µL	2,5	0,025	1,8	0,018
		5 µL	1,5	0,075	0,8	0,04
		10 µL	1,0	0,10	0,4	0,04
2 µL – 20 µL	0.5 µL – 20 µL L light gray 46 mm	2 µL	3,0	0,06	1,5	0,03
		10 µL	1,0	0,10	0,6	0,06
		20 µL	0,8	0,16	0,3	0,06
2 µL – 20 µL	2 µL – 200 µL yellow 53 mm	2 µL	5,0	0,10	1,5	0,03
		10 µL	1,2	0,12	0,6	0,06
		20 µL	1,0	0,2	0,3	0,06
10 µL – 100 µL	2 µL – 200 µL yellow 53 mm	10 µL	3,0	0,3	0,7	0,07
		50 µL	1,0	0,5	0,3	0,15
		100 µL	0,8	0,8	0,2	0,2
20 µL – 200 µL	2 µL – 200 µL yellow 53 mm	20 µL	2,5	0,5	0,7	0,14
		100 µL	1,0	1,0	0,3	0,3
		200 µL	0,6	1,2	0,2	0,4
30 µL – 300 µL	20 µL – 300 µL orange 55 mm	30 µL	2,5	0,75	0,7	0,21
		150 µL	1,0	1,5	0,3	0,45
		300 µL	0,6	1,8	0,2	0,6
100 µL – 1000 µL	50 µL – 1000 µL blue 71 mm	100 µL	3,0	3,0	0,6	0,6
		500 µL	1,0	5,0	0,2	1,0
		1000 µL	0,6	6,0	0,2	2,0
0.25 mL – 2.5 mL	0.25 mL – 2.5 mL red 115 mm	0,25 ml	4,8	12	1,2	3
		1.25 mL	0,8	10	0,2	2,5
		2,5 ml	0,6	15	0,2	5



Model	epT.I.P.S. test tip	Testing volume	Error			
			Systematic error		Random error	
			± %	± µL	± %	± µL
0.5 mL – 5 mL	0.1 mL – 5 mL violet 120 mm	0.5 mL	2,4	12	0,6	3
		2,5 ml	1,2	30	0,25	6
		5,0 ml	0,6	30	0,15	7,5
0.5 mL – 5 mL	0.1 mL – 5 mL L violet 175 mm	0.5 mL	5,0	25	1,0	5,0
		2,5 ml	3,0	75	0,9	22,5
		5,0 ml	2,0	100	0,8	40
1 mL – 10 mL	1 mL – 10 mL turquoise 165 mm	1,0 ml	3,0	30	0,6	6
		5,0 ml	0,8	40	0,2	10
		10.0 mL	0,6	60	0,15	15
1 mL – 10 mL	1 mL – 10 mL L turquoise 243 mm	1,0 ml	6,0	6	1,0	10
		5,0 ml	3,0	150	0,9	45
		10.0 mL	2,0	200	0,7	70

#### 4.1.2 Variable volume multi-channel pipette

Model	epT.I.P.S. test tip	Testing volume	Error			
			Systematic error		Random error	
			± %	± µL	± %	± µL
0.5 µL – 10 µL	0.1 µL – 20 µL medium gray 40 mm	0,5 µL	12,0	0,06	8,0	0,04
		1 µL	8,0	0,08	5,0	0,05
		5 µL	4,0	0,2	2,0	0,1
		10 µL	2,0	0,2	1,0	0,1
10 µL – 100 µL	2 µL – 200 µL yellow 53 mm	10 µL	3,0	0,3	2,0	0,2
		50 µL	1,0	0,5	0,8	0,4
		100 µL	0,8	0,8	0,3	0,3
30 µL – 300 µL	20 µL – 300 µL orange 55 mm	30 µL	3,0	0,9	1,0	0,3
		150 µL	1,0	1,5	0,5	0,75
		300 µL	0,6	1,8	0,3	0,9

#### 4.2 Maximum permissible errors in accordance with ISO 8655-2002

Nominal volume	Measurement errors			
	Systematic error		Random error	
	± %	± µL	± %	± µL
1 µL	5.0	0.05	5.0	0.05
2 µL	4.0	0.08	2.0	0.04
5 µL	2.5	0.125	1.5	0.075
10 µL	1.2	0.12	0.8	0.08
20 µL	1.0	0.2	0.5	0.1
50 µL	1.0	0.5	0.4	0.2
100 µL	0.8	0.8	0.3	0.3
200 µL	0.8	1.6	0.3	0.6
500 µL	0.8	4.0	0.3	1.5
1000 µL	0.8	8.0	0.3	3.0
2000 µL	0.8	16.0	0.3	6.0
5000 µL	0.8	40.0	0.3	15.0
10000 µL	0.6	60.0	0.3	30.0



# Evaluate Your Manual

Give us your feedback.  
[www.eppendorf.com/manualfeedback](http://www.eppendorf.com/manualfeedback)