BRAND®

APPLICATION KNOWLEDGE

User Adjustment

Adaptation to changing fluid properties

Overview

Precise pipetting requires not only high-quality pipettes, but also flexible options for adapting to changing conditions. The Transferpette® *pro* was developed precisely for this purpose: it combines high volume accuracy with simple adjustment functions so that reliable results can be achieved even under challenging conditions.

The following section explains temporary adjustment via User Adjustment and the background to volume deviations caused by special pipette tips. In addition, you will find experimental examples for specific applications at the end.

Adjustment functions of the Transferpette® *pro*

The Transferpette® *pro* microliter pipette is an air-cushion pipette with two separate adjustment functions:

Factory adjustment (Easy Calibration):
The factory adjustment is used for permanent adjustment of the devices to aqueous media in accordance with ISO 8566 in the event of volume deviations.

Temporary User Adjustment:
The User Adjustment of the Transferpette® *pro* allows temporary adjustment to conditions that deviate from the Easy Calibration adjustment (factory setting) and is therefore particularly suitable for quick adjustments to changing conditions. A safe reset to the factory setting is possible at any time and can be done quickly.

Applications:
+ Difficult liquid properties (e.g., density, viscosity, vapor pressure)
+ Temperature differences between the liquid and the environment
+ Special pipette tips

Permanent Easy Calibration technology

Temporary User Adjustment

Basics

In a microliter pipette (air cushion pipette), a piston moves a defined air cushion. This creates a vacuum that draws liquid into the pipette tip according to the volume of air moved. When the piston is moved in the other direction, liquid is expelled with the air cushion.

Pipettes are factory calibrated with water under defined conditions (ISO 8655) and are ideal for pipetting aqueous solutions.

Piston
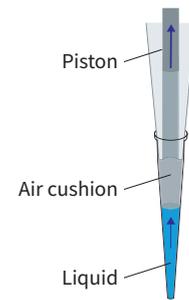
Air cushion

Liquid

Air cushion pipette

Challenge: Shape of fluid properties

In addition to pipetting technique and pipetting conditions, liquid properties that differ from those of water can also influence the pipetted volume. Challenging liquids often have several properties that differ from those of water, which can reinforce or partially offset each other.

For optimal results with challenging liquid properties, direct displacement pipettes or multiple dispensers without air cushions are generally recommended.

> **However, when using an air-cushion pipette, the pipetting results can often be improved by using a pipetting technique adapted to the liquid and additional volume compensation (User Adjustment).**

High- or low-density liquids

Pipettes are factory-calibrated for the density of distilled water. Any deviation from this density (e.g., potassium iodide solution) affects the expansion of the air cushion and thus the liquid volume. At high densities, for example, the higher hydrostatic pressure expands the air cushion, preventing it from drawing up the full volume.

> **Recommendation:**
> + Volume compensation through User Adjustment
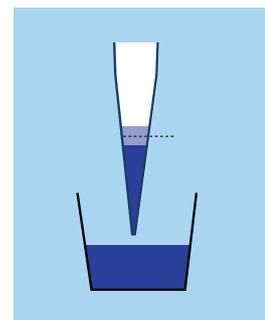
Viscous liquids

Viscous liquids (e.g., glycerol, oils) make accurate pipetting difficult because their high flow resistance slows down both aspiration and dispensing. In addition, a residual volume often remains in the pipette tip because surface wetting usually increases with these liquids.

> **Recommendation:**
> + Reverse* pipetting
> + Slow pipetting and extended waiting time
> + Volume correction through User Adjustment
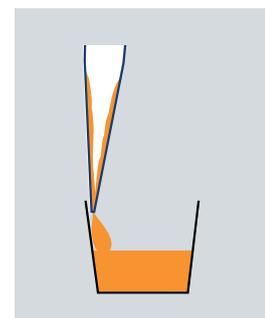
Volatile liquids

With volatile liquids (e.g., ethanol), part of the volume evaporates into the unsaturated air cushion of the pipette. This causes the air cushion to expand and pushes the liquid out of the tip. This often even leads to the formation of drops at the pipette tip.

> **Recommendation:**
> + Pre-moisten the air cushion at least 5 times by pipetting up and down (saturation of the air cushion)
> + Reverse* pipetting
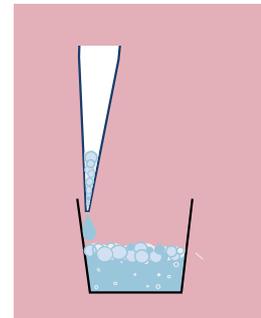> + Volume correction through User Adjustment
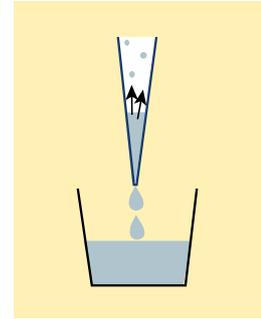
Foaming liquids

Foaming liquids make accurate and reproducible pipetting difficult. Good results can often be achieved by using an adapted pipetting technique.

> **Recommendation:**
> + Reverse* and pipette slowly.
> + If necessary, correct the volume using the User Adjustment.

## Surface-wetting liquids / liquids with low surface tension

Liquids with low surface tension tend to wet surfaces. This makes it difficult to dispense the liquid completely and evenly. Good results can often be achieved by using an adapted pipetting technique.

> **Recommendation:**
> + Use high-quality tips and reverse pipetting.
> + If necessary, correct the volume using the User Adjustment function.
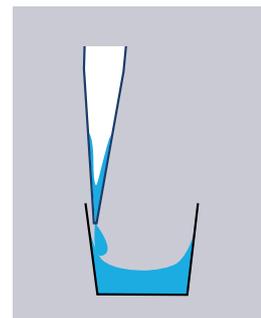
> ***ATTENTION:**
> When reverse pipetting, carefully check whether additional volume can be aspirated without the liquid coming into contact with the shaft or filter.

Temporary adjustment: User Adjustment

How to perform User Adjustment:

1. Determine volume deviation using gravimetric testing and the following simplified formulas.

$$\text{Actual volume} = \frac{\text{Mean of liquid weights}}{\text{Density of liquid} - \text{Density of air (0.0012 g/ml)}}$$

Volume offset = Target volume – Actual volume

2. Transfer or calculate the User Adjustment value from the assignment table (see below).

3. Set the User Adjustment value on the back of the device.

Example:
Pipetting 180 µl with a 20–200 µl pipette

+ Actual volume determined: 178.4 µl
+ Volume offset: 1.6 µl (=180 µl - 178.4 µl)
+ With our 200 µl device, each mark corresponds to a step value of 0.2 µl (see assignment table).
  A volume offset of 1.6 µl is added by setting +8 (= 1.6 µl / 0.2 µl).

$$\text{User Adjustment value} = \frac{\text{Step value}}{\text{Volumen offset}}$$
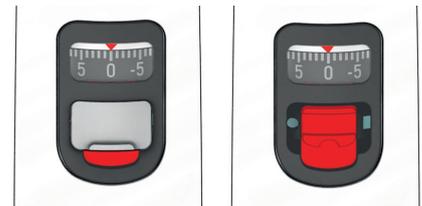
User Adjustment with closed (left) and open (right) cover

> **Note: User Adjustment per volume**
> If the User Adjustment compensates for a volume, this only applies to the selected volume. If you change the set volume, the required User Adjustment value will usually also change.

Application example: Ethanol (A), see pp. 6–7

Calculation tool

You can find a helpful calculation tool at
http://www.brand.de/uad

Setting the User Adjustment

1. Pry off and remove the cover (1) and seal (2) (e.g., using a paperclip). Dispose of the seal.

2. Slide the slider (3) down into the recess and hold it there. Use the volume-setting wheel (4) to set the desired User Adjustment value (see below) on the scale. Release the volume-setting wheel and slowly return the slider (3).

   If the slider is stuck, gently push it back toward the recess (3) and slowly return it again.

   → The value is set when the User Adjustment value aligns with the marking (5).

3. Reinsert the cover (1).

4. Verify the adjustment gravimetrically.

Set the User Adjustment

Assignment table for User Adjustment

Note: The table shows the mechanical relationship between the steps of the User Adjustment. The volume changes indicated are approximate values and apply to the entire volume range of the instrument.

					The highlighted column [1] indicates the step value for the respective instrument									
	−25	−20	−15	−10	−5	−1	0	1	5	10	15	20	25	30	35
Nominal volume µl					The step value corresponds to a volume compensation in µl:									
1	−0.025	−0.02	−0.015	−0.01	−0.005	−0.001	0	0.001	0.05	0.01	0.015	0.02	0.025	0.03	0.035
2.5	−0.05	−0.04	−0.03	−0.02	−0.01	−0.002	0	0.002	0.01	0.02	0.03	0.04	0.05	0.06	0.07
10	−0.25	−0.2	−0.15	−0.1	−0.05	−0.01	0	0.01	0.05	0.1	0.15	0.2	0.25	0.3	0.35
20	−0.5	−0.4	−0.3	−0.2	−0.1	−0.02	0	0.02	0.1	0.2	0.3	0.4	0.5	0.6	0.7
50	−1.25	−1	−0.75	−0.5	−0.25	−0.05	0	0.05	0.25	0.5	0.75	1	1.25	1.5	1.75
100	−2.5	−2	−1.5	−1	−0.5	−0.1	0	0.1	0.5	1	1.5	2	2.5	3	3.5
200	−5	−4	−3	−2	−1	−0.2	0	0.2	1	2	3	4	5	6	7
300	−6.225	−4.98	−3.735	−2.49	−1.245	−0.249	0	0.249	1.245	2.49	3.735	4.98	6.225	7.47	8.715
1000	−25	−20	−15	−10	−5	−1	0	1	5	10	15	20	25	30	35
1250	−25	−20	−15	−10	−5	−1	0	1	5	10	15	20	25	30	35
2500	−50	−40	−30	−20	−10	−2	0	2	10	20	30	40	50	60	70
5000	−125	−100	−75	−50	−25	−5	0	5	25	50	75	100	125	150	175
10000	−250	−200	−150	−100	−50	−10	0	10	50	100	150	200	250	300	350

← Volume offset for excess volume | Volume offset for missing volume →

Restore factory adjustment, reset User Adjustment

To reset the User Adjustment, set it to 0 on the scale. This restores the factory adjustment state. We recommend performing a volume check afterward.

Examples of use

All sample values were determined under the following conditions.

+ Device: Transferpette® pro 100–1000 µl (order no. 706880)
+ Volume setting: 1000 µl
+ Device, media, and room temperature: approx. 21 °C + During volume dispensing, the pipette tip was held at the first pressure point for 3 seconds.
+ After complete volume delivery (2nd pressure point for standard pipetting, 1st pressure point for reverse pipetting), the pipette tip was wiped off on the vessel wall over a distance of 10 mm.
+ Other conditions:

   A) + Tips: 50 - 1000 µl (732212)

       + No pre-conditioning of the tip: Tip change after each measurement

       + Pipetting technique: Standard (forward pipetting)

   B) + Tips: 50 - 1000 µl (732212)

       + Tips were preconditioned 5 times

       + Pipetting technique: Standard (forward pipetting)

   C) + Tips: 50 - 1250 µl (732214)

       + No preconditioning of the tip: Tip change after each measurement

       + Pipetting technique: Reverse pipetting to avoid foaming

> **Note:**
> The following examples are experimentally determined values. Since pipetting technique and environmental conditions greatly influence the determined values, we recommend determining the required correction yourself using a precision balance for the required volumes, see calculation under 'Temporary adjustment: User Adjustment, p. 2'.

Volume deviation [µl]	-25	-20	-15	-10	-5	0	5	10	15	20	25
User Adjustment	25	20	15	10	5	0	-5	-10	-15	-20	-25
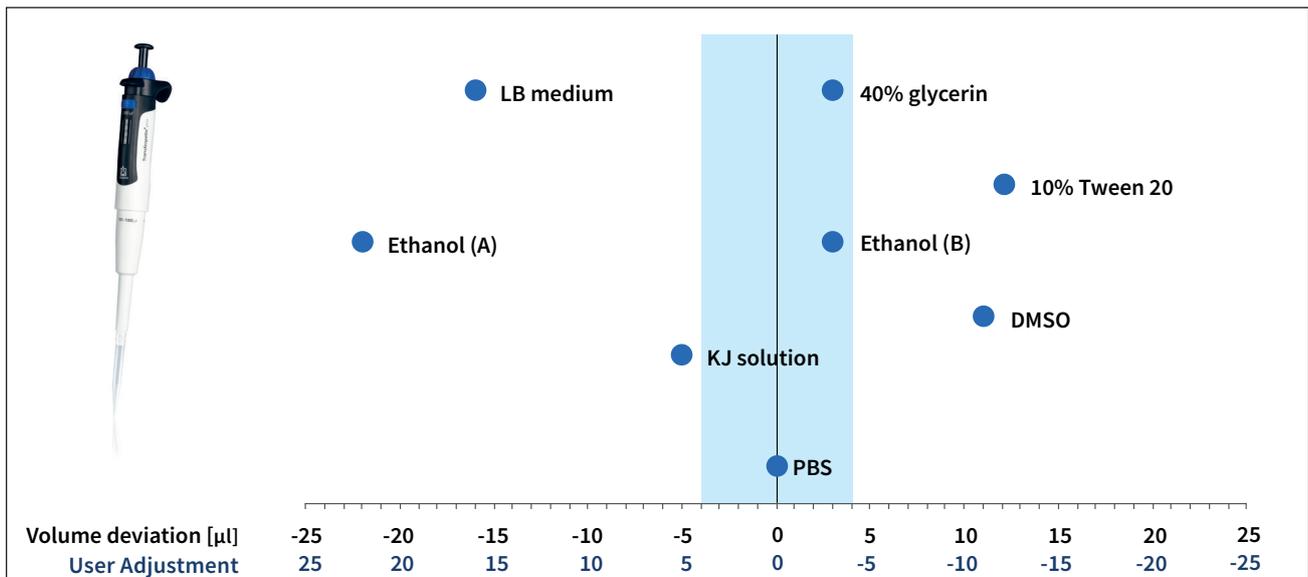
Experimentally determined User Adjustment values for a target volume of 1000 µl when using a 100-1000 µl Transferpette® pro. The pipetting conditions under which the respective value was determined can be found in the following table. For reference, the blue area indicates 50% of the accuracy tolerance according to ISO 8655 for water under standard conditions.

Liquids	Conditions	Volume deviation [µl]	User Adjustment
PBS buffer	A	0	0
Ethanol 99 %	A	-22	22
Ethanol 99 %	B	3	-3
LB medium (Lennox, 20 g/L)	A	-16	16
Potassium iodide solution (saturated)	A	-5	5
DMSO ≥ 99 %	A	11	-11
40 % Glycerin	A	3	-3
10 % Tween 20	C	12	-12

Standard PBS buffer, density: 1.00 g/ml:
 No adjustment necessary.

Ethanol 99 % (A), density: 0.79 g/ml:
Without pre-moistening/pre-conditioning, the air cushion expands due to evaporation effects and 22 µl too little is absorbed. The influence of density is overcompensated here by the influence of vapor pressure. Setting the User Adjustment to +22 compensates for the missing volume.

Ethanol 99 % (B), density: 0.79 g/ml:
After five preconditioning cycles, the air cushion is sufficiently saturated. Due to the low density and surface effects of ethanol, the air cushion expands less during volume uptake and 3 µl too much is taken up.
The excess volume can be compensated for by setting the User Adjustment to -3.

LB Medium (Lennox, 20 g/L) , density: 1,01 g/ml:
Due to wetting behavior that differs from water, liquid residues remain in the tip. This leads to a volume loss of -16 µl.
The excess volume can be compensated for by setting the User Adjustment to +16.

KPotassium iodide solution (saturated), 1.69 g/ml
The higher density of the potassium iodide solution causes greater expansion of the air cushion, resulting in 5 µl too little being aspirated.
Setting the User Adjustment to -5 compensates for the missing volume.

DMSO, density: 1.10 g/ml:
Due to the low surface tension of DMSO and the capillary forces acting in the tip, more liquid is absorbed, resulting in 11 µl too much being pipetted.
The excess volume can be compensated for by setting the User Adjustment to -11.

10 % Tween 20 in water, density: 1.01 g/ml:
To reliably pipette foaming liquids, we recommend reverse pipetting and, if necessary, the use of extended tips. In this example, extended tips (732214) and reverse pipetting resulted in a volume increase of 12 µl.
Setting the User Adjustment to -12 compensates for the excess volume.

40 % Glycerin, 1.11 g/ml
With glycerin, the capillary forces acting in the pipette tips cause an increase in volume of +3 µl. The missing volume can be compensated for by setting the User Adjustment to -3.

Further applications
You can find further technical notes on various media temperatures, peak shapes and sizes, and special media at www.brand.de.

Template documentation
User Adjustment values

You can use these form fields to document frequent User Adjustments. Enter values in the fields and simply save them. Or create your own Excel spreadsheet.

Application	Conditions*	Nominal volume	Target volume	User Adjustment
Example: XL tips		1000	100	+8

* The conditions describe your pipetting technique and external influences such as temperature, liquid, and other conceivable influencing factors.

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