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1. Introduction

The **ClearLine** single channel pipette is a volumetric instrument designed to measure and transfer liquids precisely and safely. It can measure and transfer volumes from 0.1 µl to 10000 µl depending on the model.

Model	Cat. No. Volume range [µl]	
2	257029	0.1 - 2
10	257030	0.5 - 10
20	257031	2 - 20
100	257032	10 - 100
200	257033	20 - 200
1000	257034	100 - 1000
5000	257035	500 - 5000
10000	257036	1000 - 10 000

The **ClearLine** multichannel pipettes have been designed for the filling of laboratory microplates. The pipettes enable precise and simultaneous delivery of 8 or 12 preset-volume doses of liquid.

Model	Cat. No.	Volume range [µl]
8-10 12-10	257037 257041	0.5 - 10
8-50 12-50	257038 257042	5 - 50
8-200 12-200	257039 257043	20 - 200
8-300 12-300	257040 257044	50 - 300

The volume range is shown on the pipetting pushbutton (Fig. 1A1).

The accuracy and precision (repeatability) of liquid volume depend on the quality of tips used. The values for accuracy and precision given in the table below were obtained using ClearLine tips.

Model		lume µl]	Accuracy [%]	Precision [%]	Fit to tips* [µl]
		0.2	± 12.0	± 2.8	
2		1.0	± 2.7	± 0.6	
	Max	2.0	± 1.5	± 0.4	10
	Min	0.5	± 4.0	± 2.8	10
10		5.0	± 1.0	± 0.6	
	Max	10.0	± 0.5	± 0.4	
	Min	2	± 3.0	± 1.5	
20		10	± 1.0	± 0.5	
	Max	20	± 0.8	± 0.3	
	Min	10	± 1.6	± 0.80	
100		50	± 0.8	± 0.24	200
	Max	100	± 0.8	± 0.20	
	Min	20	± 1.2	± 0.60	
200		100	± 0.8	± 0.25	
	Max	200	± 0.6	± 0.20	
	Min	100	± 1.6	± 0.40	
1000		500	± 0.7	± 0.20	1000
	Max	1000	± 0.6	± 0.15	
	Min	500	± 1.2	± 0.50	
5000		2500	± 0.6	± 0.20	5000
	Max	5000	± 0.5	± 0.15	
	Min	1000	± 2.5	± 0.6	
10000		5000	± 0.8	± 0.3	10000
	Max	10000	± 0.5	± 0.2	

* Non-filtered tip suggestion



Model	Volu [H		Accuracy [%]	Precision [%]	Fit to tips* [µl]
8-10 12-10	Min Max	0.5 5 10	±10.0 ±4.0 ±2.0	± 8.0 ± 2.0 ± 1.2	10
8-50 12-50	Min Max	5 25 50	±4.0 ±3.0 ±1.6	± 2.5 ± 1.2 ± 0.6	200
8-200 12-200	Min Max	20 100 200	±3.0 ±1.5 ±1.0	±3.0 ±1.5 ±1.0	200
8-300 12-300	Min Max	50 150 300	±1.6 ±1.2 ±1.0	± 1.5 ± 1.0 ± 0.6	300

* Non-filtered tip suggestion

The accuracy and precision are obtained, using a gravimetric method, performing at least 10 measurements of distilled water at a temperature of $20\pm1^{\circ}$ C, according to EN ISO 8655 standard.

The pipette design enables the user to perform the recalibration process according to the rules presented in section 9.

2. Setting the Volume

The pipettes are equipped with a digital counter which shows the pipetting volume. The volume setting is done by turning the pipetting pushbutton knob (Fig. 1A2) or the black adjustment knob (Fig. 1B) in the right direction.

The pipette design allows the user to lock the volume setting by pushing the locking ring (Fig.1S) upwards. The volume adjustment can be performed when the locking ring is set in the lower position (Fig.5A). When the desired volume is selected, the locking ring should be set in the upper position (Fig.5B). The position of the ring is indicated by the symbols located on the handle.

The counter displays three figures to be read from top to bottom. Additional to the figures on the lower wheel are printed graduations to enable a volume setting in the range increment of each pipette model.

Model	Counter readings	Set volume	Basic degree
2	1 2 5	1.25 µl	0.002 µl
10 20	0 7 5	7.5 µl	0.02 µl
100 200	1 2 5	125 µl	0.2 µl
1000 5000	0 7 5	0.75 ml	2 µl
10000	0 7 5	7.5 ml	20 µL
8-10 12-10	0 3 5	3.5 µl	0.02 µl
8-50 12-50	0 6 5	6.5 µl	0.10 µl
8-200 12-200	0 8 5	85 µl	0.20 µl
8-300 12-300	2 5 0	250 µl	1.0 µl

To attain the maximum accuracy, set volume must be approached from a higher value by diminishing counter readings.

 If the desired volume is lower than set volume shown by the counter, the operator should turn the pipetting pushbutton (Fig. 1A2) or the black adjustment knob (Fig. 1B) to the direction diminishing counter readings to the required volume. Before achieving the required volume slowly rotate the knob and observe carefully diminishing reading to avoid accidentally passing the setting value.



 If the desired volume is higher than set volume shown by the counter, the operator should turn the pipetting pushbutton (Fig. 1A2) or the black adjustment knob (Fig. 1B) increasing the value until the lower figure wheel comes 1/3 of a turn beyond the required setting and then slowly backward until the setting reaches the desired volume. Make sure not to pass the setting value.

If the knob is accidentally turned too far, the process must be repeated. The desired volume must always be set from the higher value in the order of decreasing value.

3. Aspirating and Dispensing

Single channel Pipettors

Place a pipette tip on the shaft of the pipettor. See section 7 for the appropriate pipette tip. Press the pipette tip on firmly using a slight twisting motion. This will ensure an airtight seal.

8-channel and 12-channel Pipettors

To put pipet tips on the shafts: hold the pipettor vertically and press it against the tips in the rack box until the shafts retract approximately 1.5 mm into the manifold. The suspension system of the shafts ensures even and tight sealing of the pipet tips. The rocking movement does not have to be performed to seal the pipet tips tightly.

Important: Never aspirate liquids into the pipette without a pipette tip attached.

Aspiration

Press the pushbutton to the first positive stop (Fig. 2A). Holding the pipette vertically, immerse the tip into the sample liquid. The depth to which the tip is immersed in the sample liquid depends on the model.

Model	Immersion depth (mm)
2, 10	≤ 1
20, 100	2 ÷ 3
200, 1000	2÷3
5000	3 ÷ 6
10000	5 ÷ 7

Release the pushbutton slowly and smoothly to aspirate the sample (Fig. 2B). Wait one second and then withdraw the tip from the liquid. When the pipette tip is immersed not as deeply as the recommended depth or when the pipetting pushbutton is rapidly released air may enter the disposable tip.

Avoid touching the orifice of the tip.

Dispensing

- Place the end of the tip against the inside wall of the vessel at an angle of 10 to 40 degrees.
- Press the pushbutton smoothly to the first stop (Fig. 2C). Wait one second.
- Press the pushbutton to the second stop to expel any remaining liquid (Fig. 2D).
- Keeping the pushbutton depressed to the very end, remove the pipette by drawing the tip against the inside surface of the receiving vessel.
- Release the pushbutton to its starting position (Fig. 2E).
- Eject the tip by pressing the tip ejector button (Fig. 2F). Remember to change the tip whenever a different kind of liquid is to be sampled.

Filters

A replaceable filter installed in a seat in the bottom part of the shaft is used in 5000 μ l and 10000 μ l pipettes (Fig. 3L). The filter prevents the aspirated liquid from entering into the shaft and thus from polluting the inside of the shaft and the piston. Using the filter is especially important when aspirating and dispensing large volumes of liquid. If the filter becomes wet during liquid aspiration it should be replaced with a new one.

4. Tip Ejector

The construction of the ejector enables the user to set up the length. The adjustable tip ejector accommodates every variety of tips available on the market. When using narrow tubes, it may be necessary to remove the tip ejector. It is simply removed by pulling down.

<u>2-1000 µl pipettes</u>

Tip ejector disassembly (Fig. 6A)

To remove the tip ejector press the tip ejector button and turn the ejector cap clockwise to the very end and next slide from the arbor.

Tip ejector assembly (Fig. 6B)

When the tip ejector button is pressed pull the tip ejector on the shaped end of the arbor to the very end and next turn counterclockwise until it is latched.



Adjusting of the Tip Ejector Length (Fig. 6C)

Adjust the tip ejector length by turning the ejector cap after removing the ejector pushbutton. To lengthen the tip ejector turn the ejector cap clockwise. To shorten the tip ejector length turn the ejector cap counterclockwise.

The 2 μ I - 10 μ I pipettes may require the use of an ejector cap "M" to efficiently eject certain brands of pipette tips. Simply place the cap, supplied with the pipette, on the bottom of the pipette shaft and slide the cap upwards until it surrounds the bottom of the tip ejector (Fig. 7A).

• 5000 and 10000 µl pipettes (Fig. 6D)

Tip ejector disassembly

Remove the tip ejector button.Use the screwdriver to turn the metal arbor left to unscrew the ejector from the arbor. Release the ejector.

Tip ejector assembly

Remove the tip ejector button. Use the screwdriver to turn the metal arbor right to screw the ejector in and adjust its length.

Tip ejector adjustment

Remove the ejector pushbutton. Use the screwdriver to increase or decrease the ejector length.

After ejector assembly or adjustment, put the ejector button back on its place.

If above described method of ejector adjustment is not sufficient or the diameter of the ejector opening is too large to eject the tip it is necessary to put the ejector cap "M" onto the ejector (Fig. 7B).

5. Pre-rising

When pipetting liquids of higher viscosity or lower surface tension than water (e.g. sera or organic solvents), a film of liquid is formed on the inside wall of the pipette tip. This film can create an error. Since the film remains relatively constant in successive pipetting operations with the same tip, this error can be avoided by forming the film before transferring the first sample. This is done by aspirating a sample and dispensing it back into the same vessel. Since the film is already formed, all of the following samples will have better accuracy and repeatability.

This pre-rinsing operation should be repeated when the volume to be aspirated is changed or when a new tip is used.

6. Dense and Viscous Liquids

The pipette specifications of accuracy and precision are based on pipetting distilled water. The handling of liquids with physical qualities of density, viscosity and surface tension differing extremely from water may need a gravimetrically checked compensation of the volume setting. Normally the degree of error resulting from heavy or viscous liquids is negligible if the pipetting is done slowly and carefully. It is most important to give the liquids some time to follow the change of pressure by holding the pipette tip in its position for at least 2 sec. after the aspiration and the blow out stroke.

If in extreme cases this method of operation does not result in accurate values, a compensation could be achieved as follows:

Weigh the liquid pipetted when the pipette is set to the nominal value. Then calculate the set-off from the nominal value:

Corr: val. = 2 x nom. val. -
$$\frac{m}{\gamma}$$

m - weight of the sample

γ – density of liquid

Check this operation once again and correct if necessary. Note the corrected value for further pipetting the same kind of liquid.

7. Pipette Tips

ClearLine tips are made from high performance polypropylene and their quality guarantees the precision and accuracy associated with the pipette. Strict control is maintained throug hout the manufacturing process to ensure the highest quality. The use of inferior quality tips will seriously degrade the performance of the pipette.

Tips 10

These tips are used for volumes between 0.1 μ l and 10 μ l. They are used with the 2, 10, 8-10, 12-10 models, which are equipped with the red pushbutton.

Tips 200

These tips are used for volumes between 2 µl and 200 µl. They are used with the 20, 100, 200, 8-50, 12-50, 8-200 and 12-200 models, which are equipped with the yellow pushbutton.



Tips 300

These tips are used for volumes between $50 \,\mu$ l and $250 \,\mu$ l. They are used with the 8-300 and 12-300 models, which are equip ped with the green pushbutton.

Tips 1000

These tips are used for volumes between 100 μ l and 1000 μ l. They are used with the 1000 model, which is equipped with the blue pushbutton.

Tips 5000

These tips are used for volumes between 500 μ l and 5000 μ l. They are used with the 5000 models, which are equipped with the white pushbutton.

Tips 10000

These tips are used for volumes between 1000 μ l and 10000 μ l. They are used with the 10000 model, which are equipped with the white pushbutton.

8. Recommendations

Observing the following recommendations will ensure maximal possible accuracy and precision of liquid sampling.

- · Make sure to operate the pipette slowly and smoothly.
- The depth of immersion in the sample liquid should be the minimum necessary and should remain constant during aspiration.
- The pipette should be held in a vertical position.
- Change the tip when volume setting is changed or when a different liquid is to be aspirated.
- Change the tip if a droplet remains on the end of the tip from the previous pipetting operation.
- Each new tip should be pre-rinsed with the liquid to be pipetted.
- Liquid should never enter the pipette shaft. To prevent this:
 - Press and release the pushbutton slowly and smoothly.
 - Never turn the pipette upside down.
 - Never lay the pipette on its side when there is liquid in the tip.
- Never force the volume setting beyond its recommended limits.
- When pipetting liquids with temperatures different from the ambient temperature, it is recommended to prerinse the tip several times before use.
- Do not pipette liquids with temperatures above 70°C.

 When pipetting acids or corrosive solutions which emit vapours, it is recommended to disassemble the shaft and to rinse the piston and seal with distilled water after finishing the pipetting operation.

9. Recalibration

ClearLine pipettes are calibrated by gravimetric method, using tips and distilled water, at the temperature 20±1°C, according to EN ISO 8655 standard.

If during pipette operation you find that the accuracy error (the difference between the real aspirated volume and the preset volume) exceeds the permissible value given in the table in section 1, the pipette recalibration procedure should be carried out.

Before starting the recalibration it is necessary to check whether the following requirements have been fulfilled during error determination:

- the ambient temperature, and the temperature of the pipette, tips and water was identical,
- the density of the liquid used is close to that of distilled water,

Volume checked [µl]	Balance sensitivity [mg]
0.1 - 10	≤ 0.001
10 - 100	≤ 0.01
> 100	≤ 0.1

- the balance with appropriate sensitivity has been used,
- mg/µl conversion factor has been taken into account,
- the requirements given in sections 3 and 8 have been fulfilled.

If the above conditions are satisfied and the accuracy error for selected volume given in section 1 exceeds the permissible value, the pipette recalibration procedure should be carried out.

The recalibration can be performed within one full turn of the key to the right or to the left only.

Recalibration conditions:

- Ambient temperature and the temperature of the pipette, tips and liquid should be within the range 20- 25°C and stabilised during weighing within ±0.5°C.
- Measurements should be conducted using distilled water.
- Balance sensitivity should be suitable for the volume to be controlled.



Recalibration procedure:

• Set the dose volume depending on the pipette volume according to the following table:

Model	Range of the pipette volumes [µl]	Preset volume [µl]	Permissible volumes [μl]	Volume change ΔV for full turn of the calibration key [μ] (24 increments)
2	0.1 - 2	0.2	0.176 - 0.224	0.06
10	0.5 - 10	0.5	0.48 - 0.52	0.33
20	2 - 20	2	1.94 - 2.06	0.63
100	10 - 100	10	9.84 - 10.16	2.50
200	20 - 200	20	19.76 - 20.24	6.30
1000	100 - 1000	100	98.4 - 101.6	25.00
5000	500 - 5000	500	494 - 506	125.00
10000	1000 - 10000	1000	975 - 1025	250.00
8-10 12-10	0.5 - 10	0.5	0.45 - 0.55	0.33
8-50 12-50	5 - 50	5	4.8 - 5.2	1.67
8-200 12-200	20 - 200	20	19.4 - 20.6	6.30
8-300 12-300	50 - 300	50	49.2 - 50.8	10.00

Single channel pipette:

 Perform 5 aspirations, weigh each one and calculate the average value of the aspirations.

Multi-channel pipette:

 Perform three aspiration series (each series should include the aspirations from all channels), weigh each time and calculate the average value of the aspirations.

Calculate average aspirated volume in [μ I] multiplying the average aspiration amount [mg] by the distilled water density coefficient [μ I/mg], which depends on temperature and pressure according to the following table:

Temperature [°C]		Pressure [kPa]	
Temperature [°C]	95.0	101.3	105.0
20	1.0028	1.0029	1.0029
21	1.0030	1.0031	1.0031
22	1.0032	1.0033	1.0033
23	1.0034	1.0035	1.0036
24	1.0037	1.0038	1.0038
25	1.0039	1.0040	1.0040

If the average aspirated volume exceeds the per missible value, the following should be done:

• Remove the pipetting pushbutton (Fig. 4A),

Warning: The pipetting pushbutton consists of 2 parts: the knob (Fig. 1A2) and the pushbutton (Fig. 1A1). After removal of the pushbutton, both parts are separated.

- Holding the volume setting knob to protect it against rotation, insert the calibration key into the cuts of the calibration screw (Fig. 4B).
- Turn the key clockwise to reduce the aspirated volume, or counter-clockwise to increase the volume (Fig. 4C). One full turn of the calibration key changes the pipette aspiration volume by the amount given in the table.
- Take out the key and fix the pipetting pushbutton (Fig. 4D). The pushbutton should be fixed by placing first the knob on the arbor (Fig. 1A2) and then the pushbutton (Fig. 1A1).
- Determine the average aspirated volume. The average volume should be within the permissible range given in the table. If the volume exceeds the values stated, the recalibration procedure should be repeated.

In case of pipetting the liquids with physical properties considerably different from those of water, follow the rules given in section 6.

10. Troubleshooting

If you notice an improper pipette operation identify the cause and eliminate the fault. Doing this, follow the instruction in the sequence provided. Replacement of elements into new ones may be required only exceptionally, and should not occur under normal pipette use.

Problem	Cause	Solution
Drealate of	The tip is emp- tied too fast.	Decrease the speed of pressing the pipette pushbutton.
Droplets of liquid remain in the pipette tip.	The tip wet- tability has increased due to extensive use.	Replace the tip with a new one.



Problem	Cause	Solution
	The pipette tip immersion is too shallow.	Immerse the tip deeper accord- ing to the instructions.
Droplets of air appear in the liquid aspirated into the tip.	The pipette tip is incorrectly pressed onto the pipette shaft.	Press the pipette firmly.
·	The tip is damaged or worn out due to extensive use.	Replace the tip with a new one.
	The pipette tip is incorrectly pressed onto the pipette shaft.	Press the pipette tip firmly.
	The shaft nut is loose (Fig. 3F) in the models 2-1000.	Tighten the shaft nut.
The pipette incorrectly aspirates the liquid or liquid drops out from the tip.	The sealing surface of the shaft is cracked or scored.	Remove the tip ejector. Un- screw the shaft nut, inspect the shaft and the piston assem- bly. Replace the damaged parts (see section 13). When reassembling the pipette, the nut should be hand tightened. In the models 10 and 20, the damage of the shaft may also cause a damage of the piston assembly. Replace the dam- aged parts (see section 13). When reassembling the pipette, the nut should be hand tightened.
	Damage of the piston or seal due to prolon ged use with the aggressive liquids.	Disassemble the pipette as described above. Replace the piston, seal and O-ring (see section 13). Rinse the inside of the shaft in distilled water and dry. Lubricate the seal and O-ring with the lub ri cant. The replacement of the piston requires conduc ting of calibra- tion procedure. Note: The parts of 2 and 10 pipette should be lubricated evenly with the minimum amount of lubricant.

Problem	Cause	Solution
	The pipette is reassembled improperly.	Disassemble the pipette and reassemble it, observing the proper sequence of steps (Fig. 3).
The pipette	No lubricant on the sealing elements.	Remove the tip ejector. Un-
incorrectly aspirates the liquid or liquid drops out from the tip.	Contamination of the inside of the pipette caused by extensive aspiration of chemically ag- gre ssive liquids or because liquid got inside the pipette.	screw the shaft nut, remove the shaft, piston assembly, seal and O-ring. Rinse the removed parts in distilled water and dry thoroughly. Lightly lubricate the inside surfaces of the seal and the O-ring with the lubricant. Reassemble the pipette in the reverse order.

Note: All parts may be autoclaved at a temperature of 121°C for 20 minutes at pressure 1 bar.

If the pipette malfunction persists after carrying out the above steps, send the pipette to your Dominique Dutscher service representative.

Before returning the pipette, make sure that it is free from any chemical, radioactive or microbiological contamination that might pose any danger during transportation and repairing. Clean the pipette as thoroughly as possible.

11. Cleaning and Sterilization

Cleaning

External surfaces of the pipetting pushbutton, the ejector pushbutton, the handgrip, the shaft nut and the adjustment knob may be cleaned using a cloth dampened in isopropyl alcohol. The remaining parts removed from the pipette during pipette disassembly may be washed with distilled water or isopropyl alcohol.

Note: Before using cleaning agents other than those recommended by the manufacturer, check the compatibility charts and consider chemical resistance of the following plastics: PP, PC, POM, PA, PPS, PVDF used to produce the pipettor parts.



Sterilization

The pipette can be sterilized in the autoclave at 121°C for 20 minutes. After sterilization, the pipette should be dried and cooled to room temperature.

It is recommended:

- to sterilize the pipettes in autoclave with an initial vacuum and drying cycle,
- prior to sterilization unscrew the shaft nut slightly in the 2-1000 μ l pipettes, and unscrew the shaft slightly in the 5000-10000 μ l. After autoclaving these parts should be screwed tight again.
- to set the locking ring in lower (unlocked) position prior to sterilization

The shaft of the 5000 and 10000 models should be autoclaved without the filter.

The precision of the results should not alter if the pipetting process and autoclaving are carried out as descri bed in this manual. Because a slight change in the accuracy of the dosage may occur, it is recommended to:

• check the calibration of the pipette after the initial first, third and fifth autoclaving cycles and then after every 10 autoclaving cycles.

Ultra Violet (UV) Sterilization

The pipettors are UV resistant. However, the recommended distance from the radiation source to the exposed element should be not less than 50 cm. Prolonged and very intense UV exposure can cause discoloration of pipettor parts without effecting their performance.

12. Pipette Kit

The pipettes are delivered in the kits including:

- Pipette
- Instruction manual
- · Calibration key
- Lubricant (single channel pipettes)
- Filters (for models 5000 and 10000)
- Ejector cap (for models 2, 10, 5000 and 10000)

13. Spare Parts and Accessories

All the spare parts and accessories indicated in Fig. 1, 3, 4:

- A: Pipetting pushbutton A1: Pushbutton A2: Knob
- B: Adjustment knob
- C: Shaft
- D: Ejector
- F: Shaft nut
- G: Piston assembly
- I: O-ring
- J: Seal
- K: Calibration key
- L: Filter
- M: Ejector cap
- N: Ejector pushbutton
- S: Locking ring

Spare parts and accessories can be ordered from your Dominique Dutscher representative (type of pipette and name of the part for this pipette should be specified).

Warning: The replacement of the piston requires conducting of calibration procedure according to section 9.