MICROLIT

MICROLIT RBO

High Precision Micropipette

Single Channel

and Multichannel

Designed and Manufactured by MICROLIT

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Operation Manual

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1. Product Description

MICROLIT RBO Micropipettes are continuously adjustable, general purpose pipettes, used for sampling and dispensing accurate and precise volumes.
They operate on the principle of air displacement (i.e. an air interface is present between the piston and the reagent/solution) and use a detachable, disposable tip. The desired volume is determined by the following formula:

$V = \pi r^2 h$

where,
V = desired volume,
π = constant (3.14),
r = radius, and
h = vertical distance travelled by the plunger.

- Single Channel Micropipettes cover volume range from 0.2 µl to 10 ml.
- Multichannel Micropipettes cover volume range from 0.5 µl to 300µl.

2. Digital Display

Fig. 2.1

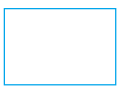
Single Channel and Multichannel Micropipettes offer easy to read Digital Displays.

3. Raw Material

The instruments are made of mechanically durable, autoclavable material.

4. Micropipette Operation

Setting the delivery volume

Sr. No.	Variable Volume Range Micropipettes	Fixed Volume Range Micropipettes
1.	Set the delivery volume using the push button on the top of the micropipette. To increase the delivery volume, turn the push button counter-clockwise. To decrease the delivery volume, turn it clockwise.	NA
2.	Make sure that the desired delivery volume clicks into place. 	NA
3.	Do not set a volume that lies outside the micropipette's specified volume range.	NA

⚠ Using excessive force to turn the push button outside the range may jam the mechanism and eventually damage the micropipette.

5. Tip Ejection

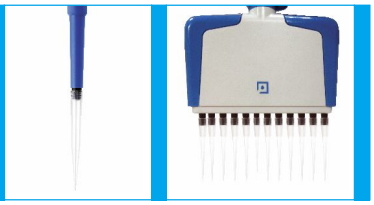
Fig. 5.1

Each pipette is fitted with a Tip Ejector Button which helps to eliminate the risk of contamination. In order to eject the tip, point the micropipette at a suitable waste receptacle and press the ejector button with your thumb.

6. Pipetting Technique

1. Press and release the push button slowly, at all times, particularly when working with high viscosity reagents/solutions.
   Do not let the push button to snap.
2. Make sure that the tip is firmly attached to the Tip Cone.

Fig. 6.1



Check for foreign particles in the tip.

3. Before you begin your actual work, fill and empty the tip 2-3 times with the reagent or solution that you will be pipetting.
4. Hold the micropipette in an upright position while aspirating. The Grippy must rest on your index finger.

Fig. 6.2

Make sure that the tips, the micropipette and the reagent/solution are at the same temperature.

7. Forward Technique

1. Fill a clean reagent/solution reservoir with the reagent/solution to be dispensed.
2. Press the push button till the first stop.

Fig. 7.1

3. Dip the tip under the upper surface of the reagent/solution in the reservoir, till a depth of about 1 cm.

Fig. 7.2

Slowly release the push button.

4. Withdraw the tip from the reagent/solution.

Fig. 7.3

Let it touch against the edge of the reservoir to remove the excess reagent/solution.

5. Deliver the reagent/solution by gently pressing the push button till the first stop. After a delay of about one second, stop.

Fig. 7.4

This action will emptythe tip.

6. Release the push button to let it retract to the ready position. If necessary, change the tip and continue pipetting.

8. Reverse Technique

The reverse technique is suitable for dispensing reagents/solutions that have a high viscosity or a tendency to foam easily. It is also recommended for dispensing very small volumes.

1. Fill a clean reagent/solution reservoir with the reagent/solution to be dispensed.
2. Press the push button till the second stop.

Fig. 8.1

3. Dip the tip under the upper surface of the reagent/solution in the reservoir, till a depth of about 1 cm. Slowly release the push button.
4. Withdraw the tip from the reagent/solution. Let it touch against the edge of the reservoir to remove the excess reagent/solution.
5. Deliver the reagent/solution by gently pressing the push button till the first stop. After a delay of about one second, continue pressing the push button all the way till the second stop. This action will empty the tip.
6. The remaining reagent/solution must either be discarded with the tip or pipetted back into the container to be dispensed.

9. Repetitive Technique

The repetitive technique offers a rapid and simple procedure for repeated delivery of the same volume.

1. Fill a clean regent/solution reservoir with the liquid to be dispensed.
2. Press the push button till the second stop. Refer to Fig. 8.1
3. Dip the tip under the upper surface of the reagent/solution in the reservoir, till a depth of about 1 cm. Slowly release the push button. This action will fill the tip.
4. Withdraw the tip from the reagent/solution. Let it touch against the edge of the reservoir to remove the excess reagent/solution.
5. Deliver the reagent/solution by gently pressing the push button till the first stop. Hold the push button at the first stop. Some liquid will remain in the tip and this must not be included in the delivery.
6. Continue pipetting by repeating step 3 and 4.

10. Pipetting of Heterogeneous Samples

Let's assume that we have to determine the deproteinization in blood glucose.

1. Use steps 1 and 2 of the forward technique to fill the tip with blood. Wipe the tip carefully with a dry and clean tissue.
2. Immerse the tip into the reagent/solution and press the push button till the first stop, making sure the tip is well below the surface.
3. Release the push button slowly to let it retract to the ready position. This will fill the tip. Keep the tip in the solution. Press till the first stop and release slowly.
   Keep repeating this procedure until the interior wall of the tip is clear.
4. Finally, depress the push button all the way to completely empty the tip.

11. Calibration and Adjustment

- All the micropipettes are factory calibrated and adjusted to give the volume as specified with distilled or deionized water, using the forward pipetting technique.
- It must be noted that the use of other pipetting techniques may affect the calibration results. The micropipettes are constructed to permit re-adjustment for other pipetting techniques or solutions/reagents of different temperatures and viscosities.

12. Device Requirements and Test Conditions

Use an analytical balance. The scale graduation value of the balance must be chosen according to the selected test volume of the micropipette.

Readable Volume Range

Graduation under 10 µl	0.001 mg
Graduation under 100 µl	0.01 mg
Graduation Above 100 µl	0.1 mg

The test liquid water is distilled or deionized "grade 3" water, conforming to ISO 3696.
All the tests are done in a draft-free room at a constant (±0.5°C) temperature of water pipette and air between 15°C to 30°C.
The relative humidity must be above 50%.
For volumes under 50 µl, the air humidity must be as high as possible to reduce the effect of evaporation.
Special accessories, such as the evaporation trap, are recommended.

Pipetting of Heterogeneous Samples

1. Repeat the pipetting step 10 times with minimum volume.
2. Again, repeat the pipetting step 10 times with maximum volume.
3. Calculate the inaccuracy (A) and imprecision (CV) for both the cases.
4. Compare the result to the limits given in the table.
5. If the calculated results are within the selected limits, the adjustments of the micropipette are correct.

Single Channel Variable Volume Micropipettes

Range	Volume µl	Inaccuracy		Imprecision	
		±%	±µl	±%	±µl
0.2-2.0 µl	2	2	0.04	1.2	0.024
0.5-10 µl	10	1	0.1	0.5	0.05
2-20 µl	20	0.8	0.16	0.4	0.08
5-50 µl	50	0.8	0.4	0.4	0.2
10-100 µl	100	0.6	0.6	0.2	0.2
20-200 µl	200	0.6	1.2	0.2	1.4
100-1000 µl	1000	0.6	6	0.2	2
500-5000 µl	5000	0.6	30	0.2	10
1000-10000 µl	10000	0.6	60	0.2	20

Single Channel Fixed Volume Micropipettes

Volume µl	Inaccuracy		Imprecision	
	±%	±µl	±%	±µl
1	5	0.05	5	0.05
2	4	0.08	2	0.04
5	2	0.1	1	0.05
10	1	0.1	0.5	0.05
20	0.8	0.16	0.4	0.08
25	0.8	0.2	0.4	0.1
50	0.8	0.4	0.4	0.2
100	0.6	0.6	0.2	0.2
200	0.6	1.2	0.2	1.4
250	0.6	1.5	0.2	1.5
500	0.6	3	0.2	1
1000	0.6	6	0.2	2
2000	0.6	12	0.2	4
5000	0.6	30	0.2	10
10000	0.4	40	0.2	20

Multichannel Micropipettes

Range	Volume µl	Inaccuracy ±%	Imprecision ±%
0.5-10 µl	1	16	10
	5	3.2	2
	10	1.6	1
02-20 µl	2	8	4
	10	1.6	0.8
	20	0.8	0.4
05-50 µl	5	8	4
	25	1.6	0.8
	50	0.8	0.4
10-100 µl	10	8	3
	50	1.6	0.6
	100	0.8	0.3
20-200µl	20	8	3
	100	1.6	0.6
	200	0.8	0.3
40-300 µl	40	8	3
	150	1.6	0.6
	300	0.8	0.3

13. Adjustment

Adjustment is done with a service tool.

1.

Fig. 13.1

Place the service tool into the openings of the calibration nut at the top of the handle.

2.

Fig. 13.2

Turn the service tool clockwise to increase, or counter-clockwise to decrease the volume

3. After adjustment, check the calibration according to the instructions given in the above section

Formula for calculating results

- Conversion of mass to volume

V= (w+e) x Z

where,
v = volume (µl),
w= weight (mg),
e= evaporation loss (mg), and
z=conversion factor for µl/mg.

- Evaporation loss can be significant with low volume. To determine the loss of mass, dispense water to the weighing vessel.

Note the reading and start a stopwatch.

See how much the reading decreases in 30 seconds (i.e. 6mg = 0.2 mg/s).

Compare this to the pipetting time from taring to reading. Typically, the pipetting time might be 10 seconds and the mass loss 2 mg (10 s x 0.2 mg/s) in this example.

If an evaporation trap or lid on the vessel is used, the correction of evaporation is usually unnecessary. The factor Z is for converting the weight of the water;
its value is 1.0032 µl/mg at 22°C and 95 kPa.

Refer to the conversion table.

Temprature °C	kPa Air Pressure						
	80	85	90	95	100	101.3	105
15	1.0017	1.0018	1.0019	1.0019	1.002	1.002	1.002
15.5	1.0018	1.0019	1.0019	1.002	1.002	1.002	1.021
16	1.0019	1.002	1.002	1.0021	1.0021	1.0021	1.0022
16.5	1.002	1.002	1.0021	1.0021	1.0022	1.0022	1.0022
17	1.0021	1.0022	1.0022	1.0022	1.0023	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024	1.0024
18	1.0022	1.0023	1.0023	1.0024	1.0025	1.0025	1.0025
18.5	1.0023	1.0024	1.0024	1.0025	1.0025	1.0026	1.0026
19	1.0024	1.0025	1.0025	1.0026	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0027	1.0028	1.0028
20	1.0026	1.0027	1.0027	1.0028	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0029	1.003	1.003
21	1.0028	1.0029	1.0029	1.003	1.0031	1.0031	1.0031
21.5	1.003	1.0031	1.0031	1.0031	1.0032	1.0032	1.0032
22	1.0031	1.0032	1.0032	1.0032	1.0033	1.0033	1.0033
22.5	1.0032	1.0033	1.0033	1.0033	1.0034	1.0034	1.0034
23	1.0033	1.0034	1.0034	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0036	1.0037
24	1.0035	1.0036	1.0036	1.0037	1.0037	1.0038	1.0038
24.5	1.0037	1.0038	1.0038	1.0038	1.0039	1.0039	1.0039
25	1.0038	1.0039	1.0039	1.0039	1.004	1.004	1.004
25.5	1.0039	1.004	1.004	1.0041	1.0041	1.0041	1.0042
26	1.004	1.0041	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0043	1.0043	1.0043	1.0044	1.0044	1.0044
27	1.0043	1.0044	1.0044	1.0045	1.0045	1.0045	1.0046
27.5	1.0045	1.0046	1.0046	1.0046	1.0047	1.0047	1.0047
28	1.0046	1.0047	1.0047	1.0047	1.0048	1.0048	1.0048
28.5	1.0048	1.0048	1.0048	1.0049	1.0049	1.005	1.005
29	1.0049	1.005	1.005	1.005	1.0051	1.0051	1.0051
29.5	1.0051	1.0051	1.0051	1.0052	1.0052	1.0052	1.0052
30	1.0052	1.0053	1.0053	1.0053	1.0054	1.0054	1.0054

14. Inaccuracy (Systematic Error)

Inaccuracy is the difference between the dispensed volume and the selected volume of a pipette.

$A = \overline{V} - V_0$

where,
A = Accuracy
$\overline{V}$ = Mean Volume
V_0 = Nominal Volume

Inaccuracy can be expressed as a relative value:

$A\% = 100\% \times A/V_0$

Imprecision (random error) - Imprecision refers to the repeatability of the pipetting. It is expressed as the standard deviation (s) or coefficient of variation (CV).

$$S = \sqrt{\frac{\sum_{i=1}^{n}(V_i - \overline{V})^2}{n-1}}$$

where,
S = Standard Deviation
$\overline{V}$ = Mean Volume
n = Number of measurement

Reproducibility or coefficient of variation (CV) can be expressedas:

$CV = 100\% \times S/\overline{V}$

15. Maintenance

- When the micropipette is not in use, make sure that it is stored in an upright position.
- We recommend a stand for this purpose.

16. Short Term Service

- The micropipette must be checked at the beginning of each day for dust and dirt on the outer surface. Particular attention must be paid to the Tip Cone.
- No other solvents except 70% ethanol must be used to clean the micropipette.

17. Long Term Service

- If micropipette is used daily, it must be checked every three months.
- The servicing procedure starts with its disassembly.

Disassembly
(For Single Channel Variable Volume Ranges 0.2µl to 200µl & Fixed Volume Micropipettes)

1.

Fig. 17.1

Press the tip ejector button and pull the ejector out.

2.

Fig. 17.2

Turn the Tip Cone counter clockwise to unscrew.

3. Fix the service tool on the O-ring seat and turn clockwise to open.

Fig. 17.3

Pull out the O-ring seat and turn the Tip Cone upside down and retrieve the O-ring.

4. Clean the Tip Cone.

5. Grease the cleaned parts with a lubricant, preferably silicon grease.

Reassembly
(For Single Channel Variable Volume Ranges 0.2µl to 200µl & Fixed Volume Micropipettes)

1.

Fig. 17.4

Place the O-ring in the Tip Cone and screw the O-ring seat with help of a service tool.

2.

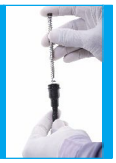


Fig. 17.5

Place the spring on the piston and slide it inside the Tip Cone.

3.

Fig. 17.6

Screw the assembled Tip Cone on the main housing.

4.

Fig. 17.7

Slide the tip ejector on the Tip Cone.

5.

Fig. 17.8

Press to fit the tip ejector.

Disassembly
(For Single Channel Variable Volume & Fixed Volume Micropipettes in the ranges 0.5-5 ml and 1-10 ml)

1.

Fig. 17.9

Pull the lower position of the ejector to dis-engage it from the upper portion.

2.

Fig. 17.10

Unscrew the Tip Cone from the mainhousing.

3.

Fig. 17.11

The Tip Cone is in two portions; the lower portion can be unscrewedfrom the upper portion to expose thepiston.

4.

Fig. 17.12

Grease the cleaned parts with a lubricant, preferably Silicon Grease.

Reassembly
(For Single Channel Variable Volume & Fixed Volume Micropipettes in the ranges 0.5-5 ml and 1-10 ml)

1.

Fig. 17.13

Screw back the lower portion of the Tip Cone on the upper part of tipcone.

2.

Fig. 17.14

Now place and screw back the spring shaft in the pipette housing by aligning the tip ejector top with the ejector pin.

3.

Fig. 17.15

Press fit the ejector top in the ejector pin firmly.

4.

Fig. 17.16

Press fit the ejector bottom.

18. Performance Optimization

Activity	Action
Consistent Technique	Pipette with a consistent rhythm, pressure and speed.
Tip Size and Fit	Use proper size tip, firmly placed on Tip Cone.
Pre-rinse Tips	Pre-rinse pipette tips for improved precision.
Sample Aspiration	Keep the disposable tip immersed in fluid during aspiration. Do not let the plunger snap back to starting position.
Immersion Depth	Maintain an immersion depth of 2 to 4 mm.
Viscous Samples	Aspirate slowly. If bubbles are observed, resample. Volume errors may still occur. Refer to the Calibration section.
Acid Samples	Pipetting strong acids and corrosive solutions is not recommended. These liquids may damage the piston and seal.
High Vapor Pressure Samples	Pipetting solutions with high vapor pressure is not recommended. These liquids may damage the piston and seal.
Sample Temperature	Fluids at a temperature other than that for which the pipette and pipette tips have been calibrated may result in volume measurement errors.
Storage	Store upright in stand. Do not lay the pipette on its side with fluid in the tip. Fluid reaching the piston causes contamination and possible corrosion.
Cleaning Piston	Wipe piston with alcohol and a soft, lint-free cloth. Dry and lightly lubricate the piston.
Performance Checks	Check the accuracy and precision of your pipette every 3-6 months depending on use and the samples aspirated.

19. Sterilization

- The micropipettes can be sterilized by autoclaving them at 121°C (252°F) at 2ata for a minimum of 20 minutes.
- No special preparation is needed.
- You may use stream sterilization bags if needed. After autoclaving, the micropipette must be cooled to room temperature for at least two hours. Before pipetting, make sure that the instrument is dry.
- We recommend that you check the calibration after every sterilization cycle to achieve the best possible precision and accuracy.

20. Troubleshooting

The table below lists possible problems and their solutions.

Single Channel Micropipettes

Problem	Possible Reason	Proposed Action
Liquid is leaking from pipette tip.	Pipette tip does not fit properly onto the tip cone.	Use the appropriate tip for exact tip fitment.
	Liquid being pipetted is hot or cold. Liquid being pipetted is very dense or viscous.	Shorten the amount of time the liquid is in the tip, or the possible solution is to use the reverse mode of pipetting.
	Pipette sealing O ring is worn.	Replace the sealing O ring.
	Tip is not loaded to maintain the seal.	Press on tip more firmly. (Avoid using too much force and over inserting the tip)
Amount of sample delivered is not accurate.	Pipette is not within calibration specifications.	Re-calibrate the pipette as per the operation Manual.
	Improper pipette technique.	See suggestions for improving pipetting technique and results.

Multichannel Micropipettes

Problem	Possible Reason	Proposed Action
Liquid is leaking from pipette tip.	Pipette tip does not fit properly onto the tip cone.	Use the appropriate tip for exact tip fitment.
	Liquid being pipetted is hot or cold. Liquid being pipetted is very dense or viscous.	Shorten the amount of time the liquid is in the tip, or the possible solution is to use the reverse mode of pipetting.
	Foreign particles between tip and Tip Cone.	Clean the Tip Cone with a lint free cloth and attach new tips.
	Tip is not loaded to maintain the seal.	Press on tip more firmly. (Avoid using too much force and over inserting the tip)
Amount of sample delivered is not accurate.	Pipette is not within calibration specifications.	Re-calibrate the pipette as per the operation Manual.
	Improper pipette technique.	See suggestions for improving pipetting technique and results.

21. Package

The micropipettes are shipped in specially designed packages containing the following items.

Single Channel Micropipettes

1. The Micropipette

Fig. 21.1

2. Service Tool

Fig. 21.2

3. Tip Sample

Fig. 21.3

4. Calibration Certificate

Fig. 21.4

5. Shelf Hanger

Fig. 21.5

6. Operation Manual

Multichannel Micropipettes

1. The Micropipette

Fig. 21.6

2. Service Tool (Fig. 21.2)

3. Tip Sample

Fig. 21.7

4. Calibration Certificate (Fig. 21.4)

5. Shelf Hanger (Fig. 21.5)

6. Reagent Trough

Fig. 21.8

7. Operation Manual

22. Caution

The micropipettes are designed to allow easy in-lab service. If you would prefer to have us or your local representative service your instrument, please make sure it has been decontaminated before you send it to us.
Please note that the postal authorities in your country may prohibit or restrict the shipment of contaminated material by mail.