CONTENTS

1. Intended use

This liquid handling device is designed and manufactured to be used with the IVD-product as an IVD-accessory and as a stand alone laboratory equipment.

2. Product description

Your new Biohit Proline®Plus is an autoclavable air displacement pipettor. The attractive and ergonomical design of the Proline®Plus pipettor together with its low pipetting forces reduce the risk of repetitive strain injuries (RSI)[1]. The pipettor is designed for both right- and left-handed use.

The special tip cone design of the pipettor offers the possibility to use replaceable Safe-Cone filters to help prevent the contamination and damage of the pipettor. The pipettor uses disposable tips.

Biohit Proline®Plus single- and multichannel pipettors

Cat. No.	Colour Code	Channels	Volume Range	Increment	Tip	Safe Cone filters	
						Standard	Plus
728010	Grey	1-ch	0.1-3 µl	0.002 µl	10	N/A	N/A
728020	Grey	1-ch	0.5-10 µl	0.01 µl	10	N/A	N/A
728030	Yellow	1-ch	2-20 µl	0.02 µl	300	721014	N/A
728050	Yellow	1-ch	10-100 µl	0.1 µl	300, 350	721008	721018
728060	Yellow	1-ch	20-200 µl	0.2 µl	300, 350	721007	721017
728070	Blue	1-ch	100-1000 µl	1 µl	1000	721006	721016
728080	Green	1-ch	500-5000 µl	10 µl	5000	721005	721015
728090	Red	1-ch	1-10 ml	20 µl	10 000	721005	721015
728515	Grey	1-ch	5 µl		10	N/A	N/A
728520	Grey	1-ch	10 µl		10	N/A	N/A
728530	Yellow	1-ch	20 µl		300	721014	N/A
728535	Yellow	1-ch	25 µl		300	721008	721018
728545	Yellow	1-ch	50 µl		300	721008	721018
728550	Yellow	1-ch	100 µl		300, 350	721008	721018
728560	Yellow	1-ch	200 µl		300, 350	721007	721017
728565	Blue	1-ch	250 µl		1000	721006	721016
728567	Blue	1-ch	500 µl		1000	721006	721016
728570	Blue	1-ch	1000 µl		1000	721006	721016
728575	Green	1-ch	2000 µl		5000	721005	721015
728580	Green	1-ch	5000 µl		5000	721005	721015
728590	Red	1-ch	10 ml		10 000	721005	721015
728120	Grey	8-ch	0.5-10 µl	0.01 µl	10	N/A	N/A
728130	Yellow	8-ch	10-100 µl	0.1 µl	300, 350	721008	721018
728140	Orange	8-ch	30-300 µl	0.2 µl	350	721007	721017
728220	Grey	12-ch	0.5-10 µl	0.01 µl	10	N/A	N/A
728230	Yellow	12-ch	10-100 µl	0.1 µl	300, 350	721008	721018
728240	Orange	12-ch	30-300 µl	0.2 µl	350	721007	721017

1. **Note:** It is knowledge that prolonged pipetting can cause RSI. The manufacturer is not responsible for RSI or any related diseases caused by prolonged pipetting.

3. Biohit tips

The full range of Biohit pipettor tips are recommended for use with Biohit Proline®Plus pipettors. Biohit standard tips are made of virgin polypropylene. Biohit also offers a full range of filter tips. Biohit standard tips are available as bulk packages, space saving refill systems and autoclavable (121°C, 20 min, 1 atm) trays. Pre-sterilized tips in trays are also available. (Fig. 1.)

Fig. 1.

4. Unpacking

The Biohit Proline®Plus pipettor package contains the following items:

1. Pipettor
2. Tip
3. Calibration tool (also tube opener)
4. Safe-Cone filters (pipettors >10 µl and forceps)
5. Grease
6. Instruction manual
7. Performance certification in accordance with ISO 8655-6

Please check that all items are included and that no damage has occurred during shipment.

5. Pipettor holder and carousel stand

For convenience and safety always keep the pipettor vertically on its own holder, carousel or linear stand when not in use.

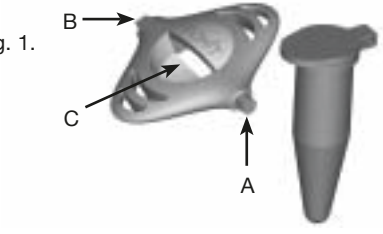
Cat.No.	Product
725600	Biohit Carousel Stand for 6 pipettors
725610	Biohit Pipettor Holder for 1 pipettor
725620	Biohit Linear Stand

6. Calibration tool (also tube opener)

The calibration tool is designed for the following purposes (Fig. 4):

1. Tool for recalibration (A).
2. Opening tool for the lid of the calibration nut (B).
3. Opening tool for the tubes (C).

Fig. 1.



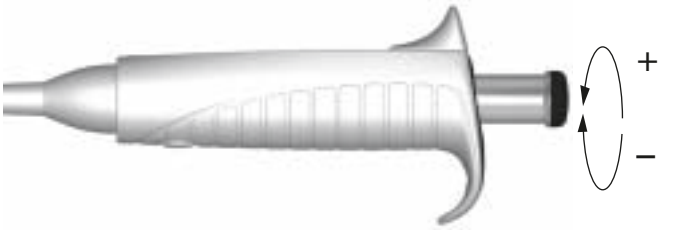
7. Operating the pipettor

7.1. Setting the volume

The volume of the pipettor is clearly shown through the display. The volume setting is carried out with the following steps (Fig. 2):

1. Set the volume by rotating the operating button (clockwise to decrease the volume and counterclockwise to increase).

Fig. 2



Note: Do not rotate the operating button outside the volume range of the pipettor (Chapter. 2.)

7.2. Sealing and ejecting tips

To ensure the maximum accuracy and precision Biohit tips are recommended to be used with Proline®Plus pipettors. Before fitting a tip ensure that the pipettor tip cone is clean. Press the tip on the tip cone of the pipettor.

Eject the tip by pressing the tip ejector with your thumb (Fig. 6). Make sure that the tip is disposed into a suitable waste container.

Fig. 3. Tip ejector

7.3. Protective tip cone filters

The tip cone design of the Biohit pipettors (>10 µl) allows the use of Biohit Safe-Cone filters in the tip cones. These removable filters prevent liquids and aerosols from entering the pipettor and, thus, protects the pipettor from contamination and damage.

The Safe-Cone filters are available as Standard or Plus versions. It is recommended to use the Standard filter for general applications and the Plus filter for more demanding applications such as cell culture, bacterial and virological work and molecular biology. Filters need to be changed regularly. The interval for filter changing is application dependant but the recommendation is to change the filter daily (after 50 - 250 pipetting cycles) and always in case of over-aspiration. (See Chapter 2 for ordering information.) Make sure that the filter is disposed into a suitable waste container. Clean the tip cone if needed and put the new filter in place.

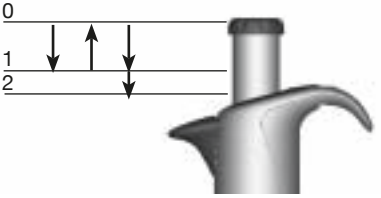
8. Pipetting techniques

Two basic pipetting techniques, forward and reverse pipetting, are associated with Proline®Plus pipettors. **Forward pipetting** is the most common used pipetting technique. The technique employs the blow-out function ensuring complete delivery of the liquid. **Reverse pipetting** is recommended for highly viscous, biological or foaming liquids, or very small volumes of liquid. A selected volume plus an excess is aspirated into the tip. The delivery is done without blow-out, and, thus, the excess volume remains in the tip. The reverse technique also facilitates the **repeated delivery** of the same volume.

8.1. Forward pipetting (Fig. 4)

1. Fit the tip onto the pipettor tip cone.
2. Press the operating button to the first stop.
3. Place the tip just under the surface of the liquid (2-3 mm) and smoothly release the operating button up to the starting position. Wait one second. Carefully withdraw the tip from the liquid, touching against the edge of the container to remove excess from the outside of the tip.
4. Dispense the liquid by pressing the operating button to the first stop. After a short delay press the operating button to the second stop. This action will empty the tip.

Fig. 4.

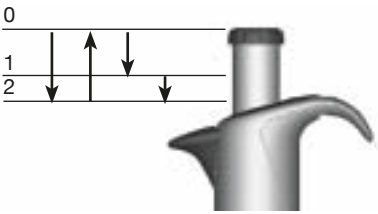


8.2. Reverse pipetting (Fig. 5)

1. Fit the tip onto the pipettor tip cone.
2. Press the operating button all the way to the second stop.
3. Place the tip just under the surface of the liquid (2-3 mm) and smoothly release the operating button up to the starting position.
4. Withdraw the tip from the liquid touching against the edge of the container to remove excess.

5. Press the operating button smoothly to the first stop to deliver the present volume. Hold the operating button at the first stop. The liquid that remains in the tip should not be included in the delivery.
6. Discard the remaining liquid by pressing the operating button to the second stop.

Fig. 5.



8.3. Repetitive reverse technique

1. Follow the reverse technique steps 1 to 5.
2. Continue pipetting by repeating steps 3 to 5 as long as needed.
3. Finally discard the remaining liquid by pressing the operating button to the second stop.

9. Recommendations for good pipetting

- Make sure that the tip is firmly attached to the tip cone.
- Hold the pipettor vertically when aspirating the liquid and place the tip only a few millimeters into the liquid.
- Always control the operating button slowly and smoothly.
- Pre-rinse the tip before aspirating the liquid by filling and emptying the tip for three to five times. This is important especially when pipetting liquids with a viscosity and density greater than water or liquids with high vapor pressure (e.g. ethanol).
- Check that the pipettor, tip and liquid are at the same temperature.
- When pipetting liquids with temperatures different to the ambient temperature change the tip after each pipetting. Do not pre-rinse the tip.
- To avoid contamination, do not rest the pipettor on its side especially with the tip attached.
- Change the tip cone filter regularly (recommendation after 50 -250 pipetting cycles).
- Never strike the tip cone against the tip tray when mounting the tip as this can damage the pipettor.
- Avoid rough handling and do not drop the pipettor.
- Avoid exposing the unit to extreme temperature changes, humidity and dust (operating temperature from 15°C to 40°C).

10. Maintenance

Biohit Proline®Plus pipettors have been designed for easy in-house service. If the pipettor is used daily it is recommended to clean/decontaminate and check the performance of the pipettor every three months. Biohit also provides complete repair and recalibration service for your pipettor including a service report and performance certificate.

Note: Please make sure that the pipettor has been decontaminated before you send it to us or your local representative. Please advise if any hazardous material has been used with your pipettor.

Note: The use of the tip cone filters may prolong the interval of the service. Change the filters regularly.

Note: It is recommended to always use gloves when cleaning the pipettor.

10.1. Daily cleaning of the outer surface of the pipettor

Your Biohit Proline®Plus pipettor should be checked every day for cleanliness. To clean and decontaminate the outer surface of your pipettor use Biohit Proline Biocontrol (Cat.no. 724004, 5 litres) and a soft lint-free cloth. It is also possible to use ethanol (70%), isopropanol (60%) or a mild detergent as a cleaning agent.
Gently clean the surface of the pipettor with moistened cloth and wipe dry. Pay special attention to the tip cone. Change the tip cone filter if needed (Chapter 7.3.)

10.2. Cleaning and decontaminating the lower part of the pipettor

If your pipettor is in daily use it is recommended to clean/decontaminate and grease the pipettor every three months. It is recommended to send the multichannel pipettors to your local Biohit service representative for cleaning and greasing. To clean and decontaminate the lower parts of the single channel pipettor, follow these steps:

Disassembling and cleaning (see the pictures under the cover page):

1. Eject the tip cone filter (if fitted, Chapter 7.3.)
2. Unscrew the tip ejector collar (1) counterclockwise and remove it.
3. Pipettors < 2000 µl:
   Unscrew the tip cone holder (2) counterclockwise and carefully remove it with the tip cone (3).

   Pipettors 2000, 5000, 10 000 µl:
   Unscrew the tip cone cylinder (4) counterclockwise and remove it.
4. Clean the tip ejector collar, the tip cone holder, the tip cone (cylinder) and the piston (5) with Biohit Proline Biocontrol, ethanol (70%), isopropanol (60%) or mild detergent and soft lint-free cloth.
5. Clean the interior of the tip ejector collar and the tip cone (cylinder) with a cotton swab. Be careful with the pipettors so that the seal inside the tip cone will not damage.

6. Rinse the parts with distilled water if needed and let the parts dry.
7. Pipettors ≤ 100 µl:
   Put a thin layer of grease on the piston (5).

   Pipettors 250, 500 and 1000 µl:
   Put a thin layer of grease around the seal (6).

   Pipettors 2000, 5000 and 10 000 µl:
   Put a thin layer of grease on the seal (6) and the interior of the tip cone cylinder (4).

Note: Avoid excess grease. Use only the grease provided with the pipettor.

Note: Before reassembling check that no lint or particles are on the surface of the piston.

Decontamination:

For complete decontamination of the lower parts place the tip ejector collar (1), tip cone holder (2), tip cone (3) and tip cone cylinder (4) (only the models 2000, 5000, 10 000) into a beaker containing Biohit Proline Biocontrol and leave for at least 30 minutes. Wipe the piston with Biohit Proline Biocontrol and the lint-free cloth. Rinse the parts with distilled or sterile water. Let the parts dry. Grease the piston and seal according to the instructions given earlier in this chapter.

Reassembling:

1. Pipettors:
   Carefully place the tip cone (3) on the piston and attach it by screwing the tip cone holder (2) clockwise.

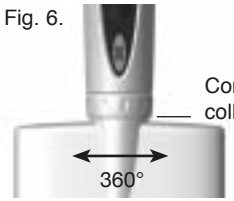
   Pipettors 2000, 5000 and 10 000 µl:
   Carefully place the tip cone cylinder (4) on the piston and screw clockwise. Make sure that the tip cone (cylinder) is properly tightened. Avoid over tightening.
2. Attach the tip ejector collar (1) by screwing it clockwise.
3. Place the new tip cone filter on its place.
4. Press the operating button several times to ensure that the grease has spread evenly.
5. Check the performance of the pipettor.

Note: It is always necessary to check the performance of the pipettor after in-house service or maintenance.

10.3. Sterilization of the pipettor

The entire Proline®Plus pipettor can be sterilized by steam autoclaving at 121°C, (252°F), 1 atm for 20 minutes. Remove the tip cone filter (if fitted, Chapter 7.3). The single channel pipettors can be autoclaved without special preparations. **Unscrew the lower part of the multichannel pipettors by holding the connecting collar and turning the lower part 360° counterclockwise (Fig. 6).** Put the pipettor into the sterilization bag and place it into the autoclave. After autoclaving the pipettor must be

Fig. 6.



cooled down and left to dry overnight before use. Screw the lower part of the multichannel pipettors by holding the connecting collar and turning the lower part 360° clockwise. Make sure that the lower part is properly tightened. It is recommended to check the performance of the pipettor after every autoclaving. It is also recommended to grease the piston/seal of the pipettor after every 10 autoclaving.

11. Testing the performance and recalibration

It is recommended to check the performance of your Biohit Proline®Plus pipettors regularly (e.g. every 3 months) and always after in-house maintenance. However, the user should establish a regular testing routine for their pipettors with regard to accuracy requirements of the application, frequency of use, number of operators using the pipettor, nature of the liquid dispensed and the acceptable maximum permissible errors established by the user. (ISO 8655-1.)

11.1. Testing the performance

Performance testing should take place in a draught-free room at 15 - 30°C, constant to +/- 0.5°C and humidity above 50%. The pipettor, tips and the test water should have stood in the test room a sufficient time (at least 2 hours) to reach equilibrium with the room conditions. Use distilled or deionised water (grade 3, ISO 3696). Use an analytical balance with a readability of 0.01 mgs. (ISO 8655-6.)

Weighing

1. Adjust the desired test volume V_s.
2. Carefully fit the tip onto the tip cone.
3. Fill the tip with test water and expel to waste five times to reach a humidity equilibrium in the dead air volume.
4. Replace the tip. Pre-wet the tip by filling it once with test water and expel to waste.
5. Aspirate the test water, immersing the tip only 2-3 mm below the surface of the water. Keep the pipettor vertical.
6. Withdraw the pipettor vertically and touch the tip against the inside wall of the test water container.
7. Pipette the water into the weighing vessel, touching the tip against the inside wall of the vessel just above the liquid surface at an angle of 30° to 45°. Withdraw the pipettor by drawing the tip 8-10 mm along the inner wall of the weighing vessel.
8. Read the weight in mgs (m_i).
9. Repeat the test cycle until 10 measurements have been recorded.
10. Convert the recorded masses (m_i) to volumes (V_i)

    $V_i = m_i Z$ $\qquad$ Z = correction factor (Table 1)
11. Calculate the mean volume ($\overline{V}$) delivered:
    $\overline{V} = (\sum V_i)/10$
12. For conformity evaluation calculate the systematic error e_s of the measurement:
    in µl: $e_s = \overline{V} - V_s$ $\qquad$ V_s = selected test volume

    or in %: $e_s = 100\ (\overline{V} - V_s)/V_s$

13. For conformity evaluation calculate the random error of the measurement:

    as standard deviation $s = \sqrt{\frac{\sum(V_i - \overline{V})^2}{n - 1}}$ n = number of measurement (10)

    or as coefficient of variation $CV = 100s/\overline{V}$
14. Compare the systematic error (inaccuracy) and random error (imprecision) with the values in the performance specifications - (p. 49) or the specifications of your own laboratory.
    If the results fall within the specifications, the pipettor is ready for use. Otherwise check both systematic and random errors and, when necessary, proceed to the recalibration procedure (Chapter 11.2).

Note: Systematic error (inaccuracy) is the difference between the dispensed volume and the selected test volume. Random error (imprecision) is the scatter of the dispensed volumes around the mean of the dispensed volume. (ISO 8655-1.)

Note: Biohit specifications are achieved in strictly controlled conditions (ISO 8655-6). The user should establish own specifications based on the field of use and the accuracy requirements placed on the pipettor (ISO8655-1).

Table 1

Z-values (µl/mg):				
Temp. (°C)	Air Pressure (kPa)			
	95	100	101.3	105
20.0	1.0028	1.0028	1.0029	1.0029
20.5	1.0029	1.0029	1.0030	1.0030
21.0	1.0030	1.0031	1.0031	1.0031
21.5	1.0031	1.0032	1.0032	1.0032
22.0	1.0032	1.0033	1.0033	1.0033
22.5	1.0033	1.0034	1.0034	1.0034
23.0	1.0034	1.0035	1.0035	1.0036
23.5	1.0036	1.0036	1.0036	1.0037

Note: This method is based on ISO 8655.

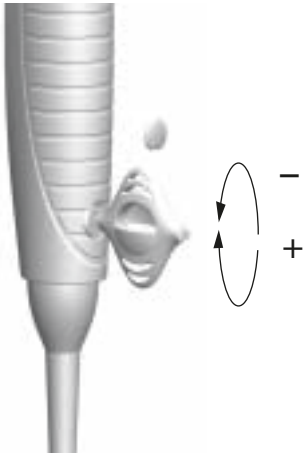
11.2. Recalibration

The calibration of your Proline®Plus pipettor has been factory checked and certified at 22°C using grade 3 distilled water according to ISO 3696. The calibration is based on ISO 8655-6: Gravimetric test method for volumetric instruments. The pipettor's specifications are guaranteed only with genuine Biohit tips. If you find the pipettor to be inaccurate after performance testing, please follow the instructions below:

1. Remove the lid of the calibration nut, located at the backside of the handle, with the aid of the calibration tool (Fig. 7).
2. Place the hexagonal head of the calibration tool into the hole of the calibration nut.
3. Turn the adjustment lock counterclockwise to decrease and clockwise to increase the volume.
4. Repeat testing the performance procedure (Chapter 11.1.). Continue until the results are correct.

Note: Biohit offers accredited calibration service. Please contact your local Biohit representative for further information.

Fig. 7.



12. Trouble shooting

Symptom	Possible cause	Solution
Droplets left inside the tip	Unsuitable tip	Use original tips
Leakage or pipetted volume too small	Non-uniform wetting of the plastic	Attach new tip
	Tip incorrectly attached	Attach firmly
	Unsuitable tip	Use original tips
	Foreign particles between tip and tip cone	Clean the tip cone, attach new tip
	Tip cone holder incorrectly tightened	Tighten the tip cone holder
	Pipettor damaged	Return to your Biohit service representative for servicing
Pipettor out of established specifications	Incorrect operation	Follow instructions
	Unsuitable tip	Use original tips
	Calibration altered	Recalibrate
Operating button jammed or moves erratically	Liquid has penetrated tip cone and dried	Clean and grease the piston/seal and the tip cone
	Safe Cone filter has been contaminated	Change filter
	Insufficient amount of grease on a piston and seal	Grease accordingly
Tip ejector jammed or moves erratically	Tip ejector collar has been contaminated	Remove and clean the ejector collar and tip cone

13. Warranty information

The Biohit Proline®Plus pipettors are covered by warranty for 3 years against defects in materials and workmanship. Should your pipettor fail to function at any time, please contact your local Biohit representative.

ANY WARRANTY WILL, HOWEVER, BE DEEMED AS VOID IF FAULT IS FOUND TO HAVE BEEN CAUSED BY MALTREATMENT, MISUSE, UNAUTHORIZED MAINTENANCE OF SERVICE OR NEGLIGENCE OF REGULAR MAINTENANCE AND SERVICE, ACCIDENTAL DAMAGE, INCORRECT STORAGE OR USE OF THE PRODUCTS FOR OPERATIONS OUTSIDE THEIR SPECIFIED LIMITATIONS, OUTSIDE THEIR SPECIFICATIONS, CONTRARY TO THE INSTRUCTIONS GIVEN IN THIS MANUAL OR WITH OTHER THAN THE MANUFACTURER'S ORIGINAL TIPS.

Each Biohit Proline®Plus pipettor is tested before shipping by the manufacturer. The Biohit Quality Assurance Procedure guarantees that the Biohit Proline®Plus pipettor you have purchased is ready for use.

Each Biohit Proline®Plus pipettor is CE marked.

14. Performance specifications

The manufacturer's specifications (p. 49) are guaranteed only when the manufacturer's original tips are used. The manufacturer's specifications should be used as guidelines when establishing your own performance specifications in accordance with ISO 8655.