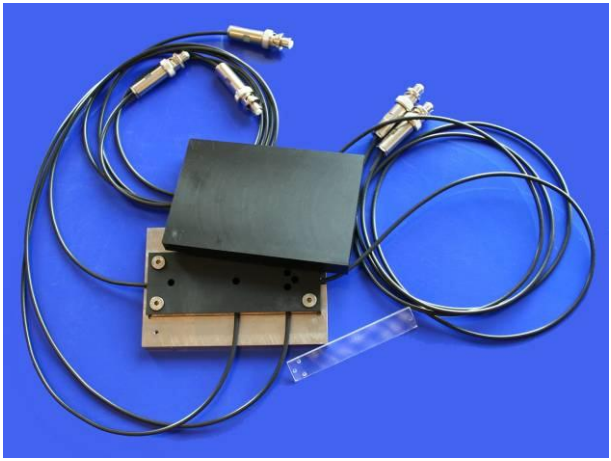
micronit

MICROFLUIDICS

MICROFLUIDIC EOF KIT 9015

User Manual - Version 1.1



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ELECTRICAL SAFETY

The chip holder in this kit has been designed for use with a high voltage supply. High voltage equipment should only be handled by suitably trained personnel. Do not use voltages higher than 6 kV. Always check that the microchip has been properly installed before starting the analysis. Fluid leaking through the o-ring seal can expose the user to dangerous voltages and can cause permanent damage to attached electrical equipment. Micronit cannot be held responsible for damage done to electrical equipment. Always use the shipped interlock cable which disables the high voltage power supply when there is no cover on the holder. Do not pull on the cables when unplugging the high-voltage connectors.

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1 INTRODUCTION

This manual describes the installation and use of the Microfluidic EOF Kit 9015. The kit has been developed specifically for use with Micronit chips without integrated electrodes.

The Microfluidic EOF Kit 9015 can be used with a LabSmith high voltage sequencer or compatible high voltage power supply. Low background fluorescence of the holder enables fluorescence detection and the layout of the microchip remains flexible using a standardized hole pattern.

A window in the bottom of the holder enables visual inspection of the entire channel. This is especially valuable for method development (observing the sample plug formation and dispensing with an inverted microscope) and required for optical detection.

The chip holder is especially suitable for applications requiring fluorescence detection. Electrochemically inert platinum wires are integrated in the reservoirs for applying the high voltage.

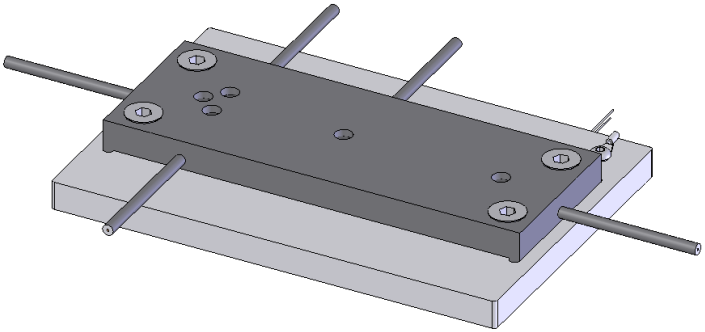


Figure 1.1: Schematic drawing of the chip holder EOF 9015

Key features of the Microfluidic EOF Kit 9015:

- User friendliness
- Low background fluorescence
- Chemical inert wetted materials (microchip, seals and holder)
- Optical inspection of the microfluidic channels under a microscope possible
- Rigid stainless steel bottom plate

2 PARTS LIST

Please check if all the following parts are included in the kit.

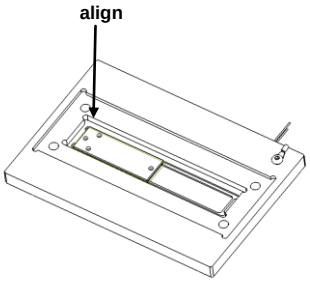
- **1** Assembled chip holder (consisting of a bottom plate, top plate and cover)
- **5** High voltage cables with SHV-BNC connectors
- **1** Safety interlock cable with BNC connector
- **5** Replacement O-rings
- **1** Hex head wrench 2
- **1** Hex head wrench 3
- **1** Manual
- **1** Syringe with Luer tip (2.5 mL)
- **1** Glass microfluidic chip T8050
- **1** Glass microfluidic chip X3550

3 INSTALLING A MICRONIT CHIP

The chip holder has been designed for microchips with a maximum size of 15x90 mm and a thickness between 1.0 mm and 2.0 mm. To install a microchip place the bottom plate on a flat surface. Place a microchip in the recess in the bottom plate and make sure the microchip is level and sits against the left edge (see figure). The microchip should be placed in such a way that the three fluidic holes face upwards and are on left hand side.

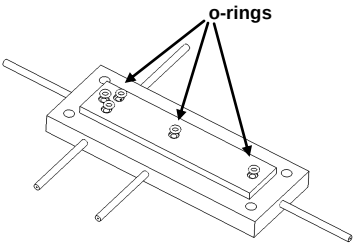
Closing the chip holder

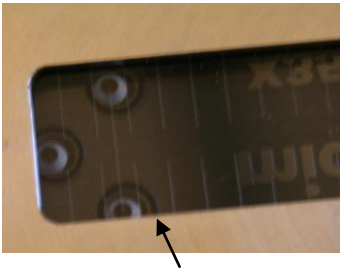
Take the top of the holder and check that all o-rings are in place. The cover should be completely dry before assembling. Position the top of the holder on the bottom plate aligning the fluidic holes on the microchip with the reservoirs in the top of the holder. Apply an even pressure on the top of the holder and making sure that the top is level. Tighten the 4 hex head bolts using the supplied 3 mm hex head wrench. Be careful not to over tighten the bolts.



Inspection

Turn the holder upside down and inspect all o-ring seals. When looking under an angle a concentric bright black ring can be observed where the o-ring contacts the glass, indicating a good seal (see figure). When using microchips smaller than 90x15 mm (for instance 45x15 mm) make sure that the holes on the microchip are properly aligned with the reservoirs in the top of the holder.





Black ring indicating a good seal

4 FILLING THE CHIP WITH ELECTROLYTE

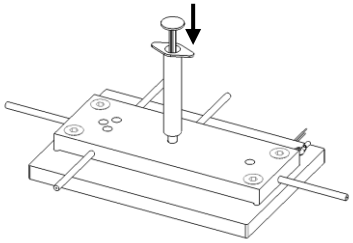
The easiest way to fill the compartments is using a micropipette (most yellow 2-200 µL tips with a sharp point reach to the bottom of the microchip). The compartments can hold between 20 and 150 µL of fluid, but preferably 50 to 120 µL is used. Dispense an amount of electrolyte in the outlet compartment. The electrolyte will spontaneously fill the channels by capillary action. This will take approximately 20 seconds for an aqueous solution and a clean microchip (X3550). Fill the remaining compartments with electrolyte. Dispense the same amount of fluid in all wells to prevent siphoning which would lead to zone broadening during capillary electrophoresis experiments. Usually, it is not necessary to use degassed electrolytes. Place the cover on the holder.

Air bubbles

When a microchip is installed that has been used before, it is possible that air bubbles get trapped inside the microfluidic channels when only the capillary action is used for filling. It is therefore important to check whether the channels are properly filled (e.g. visually or by monitoring the electrical current). Air bubbles can be removed by flushing the microchip with the supplied syringe.

4.1 FLUSHING THE MICROCHIP

In order to flush the microchip, fill at least one of the compartments with a maximum of 50 µL of liquid. Pull back the plunger of a Luer tip syringe (a 2.5 mL syringe is supplied with the kit) and insert the syringe in one of the conical compartments. Press on the plunger to compress the air and hold it to flush the microfluidic channels. Do not use excessive pressure as this can force liquid past the o-ring seals. It is also possible to use a vacuum to flush the microchip, but positive pressure generally is more effective, especially when removing air bubbles. Sometimes air bubbles remain stagnant in the channels but will gradually dissolve in the liquid flowing past them aided by the increased pressure. In contrast, when using vacuum there is a risk that air bubbles are generated inside the channels when the electrolyte has not been degassed first.



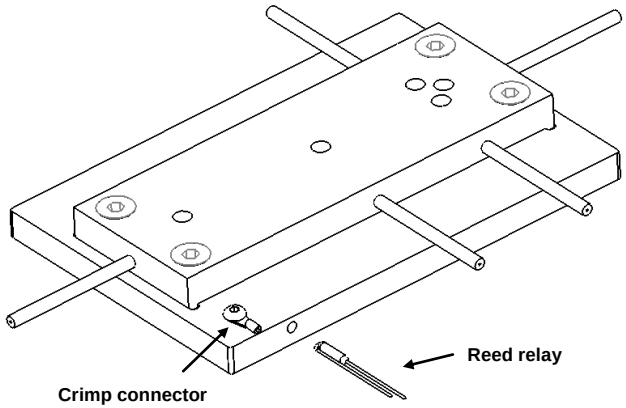
Always place the cover on the holder in order to prevent accidental contact with the electrolyte when the high voltage is on and to prevent dust collecting in the fluidic compartments.

5 ELECTRICAL CONNECTIONS

The Microfluidic EOF Kit 9015 is supplied with five high voltage cables with SHV-BNC connectors. Attach the cables to the high voltage supply output. The cable with the BNC connector should be connected to the interlock input of the high voltage supply. When using this safety feature the high voltage supply automatically switches off the output when the cover is removed from the chip holder during an experiment. The reed relay can be pulled out of the holder for inspection if needed.

Grounding

For electrical safety reasons the user is advised to connect the metal base plate to an electrical ground. A crimp connector on the corner of the holder can be used for this purpose.



6 CAPILLARY ELECTROPHORESIS SEPARATION

A capillary electrophoresis (CE) separation generally starts with the formation of a sample plug in the channel cross or double-T, followed by the actual separation step. An example of typical voltage sequences that can be used to generate a sample plug in an X3550 chip by means of a gated injection is shown in table 1.

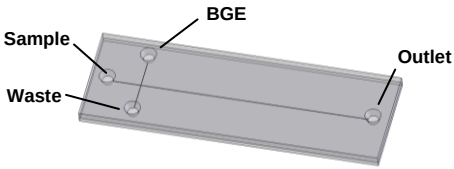


Table 1: *Example of a voltage sequence for a gated injection on an X3550 chip.*

	Sample (V)	*BGE (V)*	*Waste (V)*	*Outlet (V)*
Separation	900	1000	300	0
Gating	900	600	300	0

Before applying the high voltage, make sure that there is no liquid leaking out of the reservoirs. Also make sure that all electrical connections are securely fastened.

The electrical field strength $E_{1...4}$ [V/cm] inside the 4 channels of a cross channel chip can be calculated based on the voltage applied $V_{1...4}$ [V] and the channel length between sample cross and inlet/outlet compartments $L_{1...4}$ [cm] using the equation:

$$E_n = \frac{V_n}{L_n} - \frac{L_2L_3L_4V_1 + L_1L_3L_4V_2 + L_1L_2L_4V_3 + L_1L_2L_3V_4}{L_n(L_1L_2L_3 + L_2L_3L_4 + L_1L_3L_4 + L_1L_2L_4)}$$

This equation is valid when the electrical conductivity of the background electrolyte is equal in all parts of the channel. The sign of the electric field denotes the direction.

The channel lengths for standard chips (X3550 or T8050) are provided in table 2.

Table 2: *Channel length between sample cross and inlet/outlet compartments*

	X3550	***T8050***
L_1	4.3 mm	4.3 mm
L_2	4.3 mm	4.3 mm
L_3	4.3 mm	4.3 mm
L_4	34 mm	79 mm

7 CLEANING

The chip holder and the microchips can be cleaned according to the procedure described below.

7.1 CLEANING THE CHIP HOLDER

Before removing the microchip, empty all compartments in the chip holder and fill them with deionized water and flush the microchip using the syringe as described in section 4.1. When salt solutions are allowed to dry out inside the microchip, the channels can become blocked completely by crystals making it difficult to fill the microchip with electrolyte the next time. The reservoirs can now be emptied and the holder can be disassembled by undoing the four screws.

In order to completely clean the top of the holder start by removing the o-rings on the bottom side. The o-rings and the top of the holder can be cleaned using deionized water. Make sure the cover is completely dry before reassembling the holder again.

7.2 CLEANING THE MICROCHIPS

During use, the channel surfaces of the microchips may become covered with adsorbed organic contamination. This often results in a gradual decrease of the electroosmotic flow. In order to clean the channels and restore the electroosmotic flow, the microchips can be cleaned by flushing with up to 1 mol/L sodium hydroxide. It is best to do this with the microchip removed from the chip holder since this will prevent problems with cleaning the holder and interference of the cleaning agent with subsequent analyses. Short term exposure to alkaline cleaning solutions will not damage the holder. Thoroughly rinse the microchip with deionized water afterwards.

Caution: do not expose the holder to concentrated sodium hydroxide (i.e. >50%) or concentrated acids. The microchips can be safely flushed with 1 mol/L sodium hydroxide for cleaning. If unsure, consult a datasheet to check the chemical compatibility of POM.

8 FREQUENTLY ASKED QUESTIONS

Electrolyte leaks out of the compartments

Cause: The microchip is not sitting horizontal in the bottom part of the holder, the four screws are not tightened sufficiently or too much on one side only.
Solution: Remove the top, thoroughly dry everything and reassemble.

Cause: The compartments on the microchip are not properly aligned with the top of the holder.
Solution: Remove the top, thoroughly dry everything and reassemble. Make sure the microchip sits against the edge of the recess. Inspect by looking from the bottom whether the o-rings make a good seal (see p. 6)

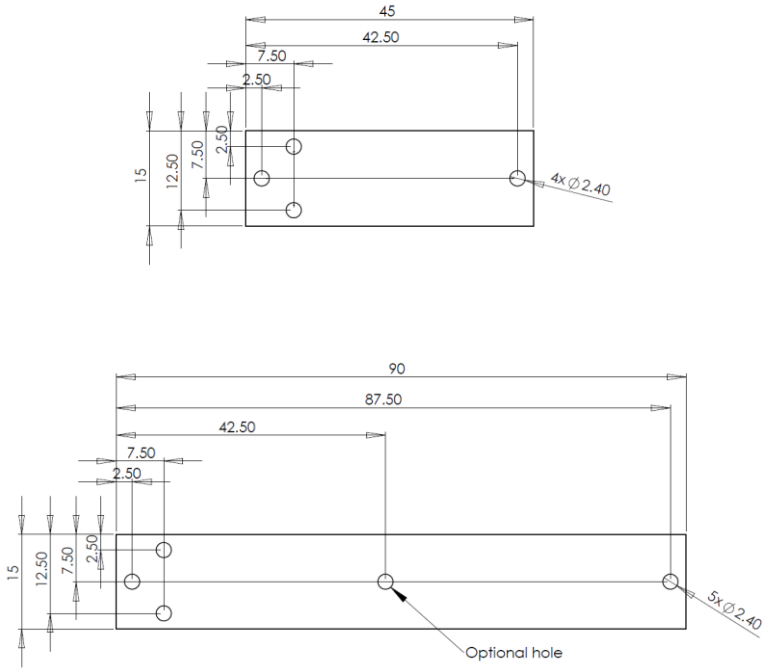
The high voltage cannot be switched on

Cause: The reed relay is not aligned properly to the magnet in the cover or it is broken.
Solution: Pull out the reed relay and check that is has not been damaged. Reinsert the reed relay.

9 DESIGN YOUR OWN MICROCHIPS

You can design your own customized microchips for the Microfluidic EOF Kit 9015.

Use the dimensions shown below. The fluidic holes are optional.



10 TECHNICAL SPECIFICATIONS EOF 9015

Chip Holder dimensions (without cables)

127 x 85.5 x 28 mm (suitable for use with microscopes accepting microtiter plates)

Distance underside of the holder to the underside of the microchip

1.0 mm

Materials

Stainless steel bottom plate, black POM top plate and cover.
Maximum continuous operating temperature: 80°C

Wetted materials

POM (chip holder), Viton (o-rings), platinum wire electrodes, borosilicate glass (CE microchip)

Microfluidic chips

Compatible with microchips with a maximum size of 90 x 15 mm. Thickness 1.0 to 2.0 mm.

- Fluid capacity of the wells : 150 µL
- Minimum volume required in the wells : 20 µL
- Recommended sample volume : 50 – 120 µL

Electrical connections

Five high voltage cables (tested for 17 kV DC) are attached to the holder. The cables are supplied with SHV-BNC plugs that can be connected directly to a LabSmith high voltage sequencer (www.labsmith.com) or compatible high voltage power supply. The length of the cables is 1 m.

Maximum separation voltage

The maximum separation voltage is 6 kV.

Electrical safety

The integrated safety interlock switch ensures that the high voltage sequencer cannot be started without the cover placed on the holder (in combination with a LabSmith high voltage sequencer or compatible high voltage power supply with an interlock feature). All voltage carrying parts are shielded from the user.