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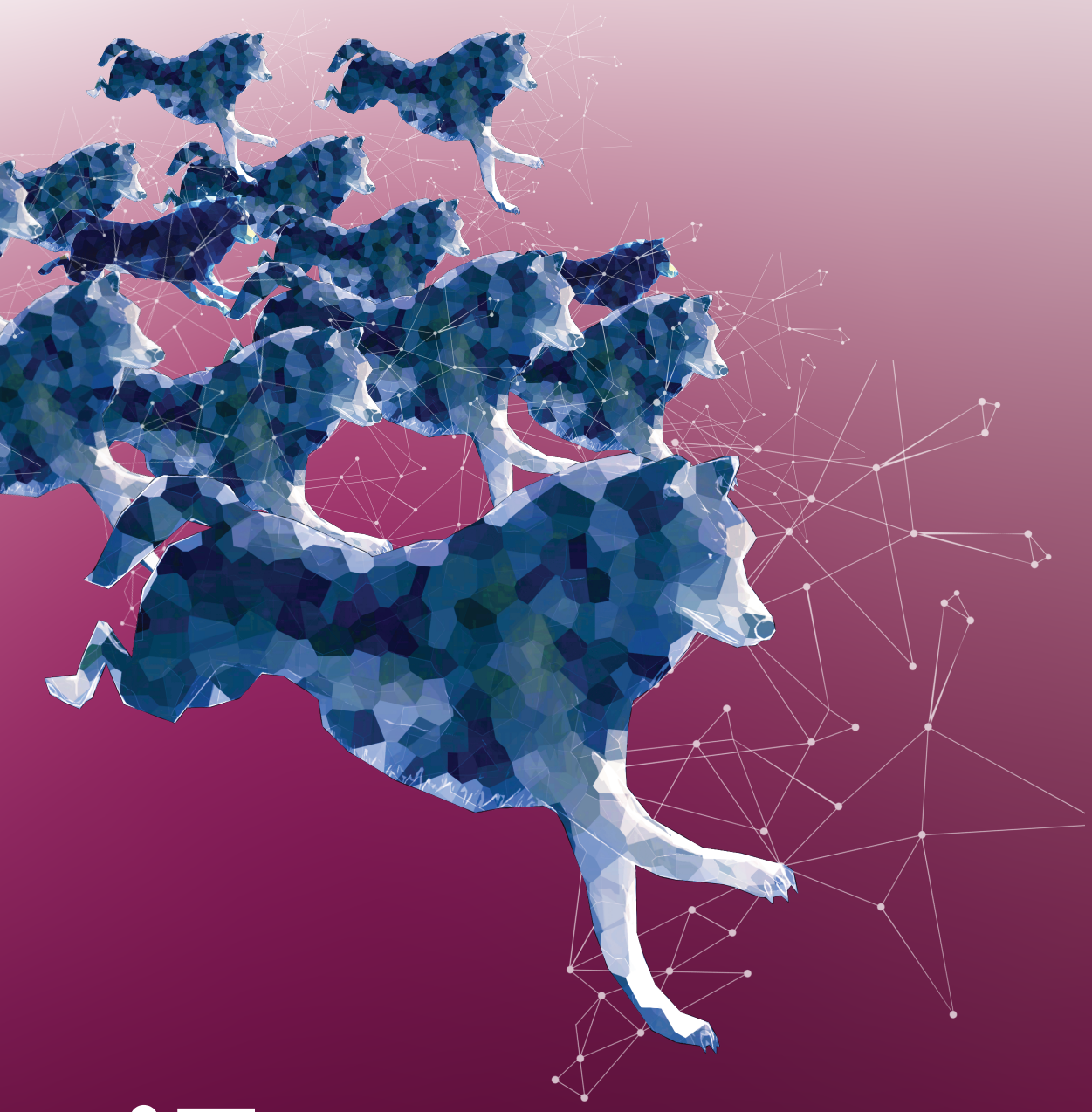

siFractor

Precise, Simple & Semi-automated Fractionation

For the online manual visit:
https://sitoolsbiotech.com/protocols.php

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siFractor Manual

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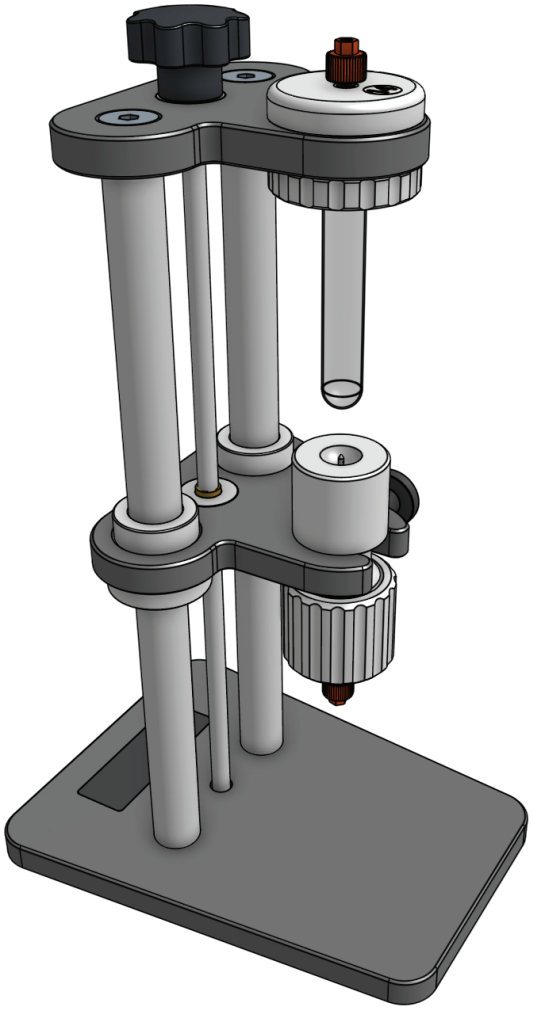

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Product contents

siFractor

The siFractor is a device for fractionation of samples contained within polypropylene tubes typically used in centrifugation applications. Different sample holders are available for tubes with diameters of 11 mm (SW60), 13 mm (SW55), 14 mm (SW40/41) or 25 mm (SW28). See **Supplementary Materials Table 1** for detailed rotor information.

Product Components

Components	Quantity
siFractor unit (consisting of stand, sample holder and piercing unit)	1
O-ring set (**Supp. Mat. Table 2**)	
Union M6	2
Sample holder	2
Silicone grease	1
Tools (for needle, luer lock and needle socket)	3
Union 1/16" Female to M6 Male	2
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Required Instrumentation & Compatibility

The siFractor is operated by a HPLC or FPLC machine (**host system**). It is connected to the system instead of a column.

- The siFractor is connected to the host system via O-ring sealed **M6 threads**. Unions are provided for **1/16" fingertight** connectors that are typically used in commercially available ÄKTA systems. For all other types of connections, unions or adapters need to be supplied by the user.
- When using viscous solutions for fractionation (e.g. sucrose solutions >40% w/v), it is recommended to equip the host system with **tubing with an inner diameter of at least 0.75 mm** to reduce the pressure in the system.
- It is recommended to use a **super loop (or similar device)** to deliver the chase solution. The system pump(s) can thus be operated with water, minimizing time spent on maintenance and cleaning of the host system.
- **Centrifugation tubes** need to be supplied by the user. Prior to centrifugation, make sure that the tubes snugly fit into the sample holder. They should engage both o-rings for proper sealing. If excessive force needs to be applied to insert the tube into the sample holder, or if, after insertion, it slips out of it easily, it is not compatible. In this case contact customer support for assistance.
- O-rings are made from **silicone (MVQ)** and should not be replaced by the more commonly available NBR or other materials.

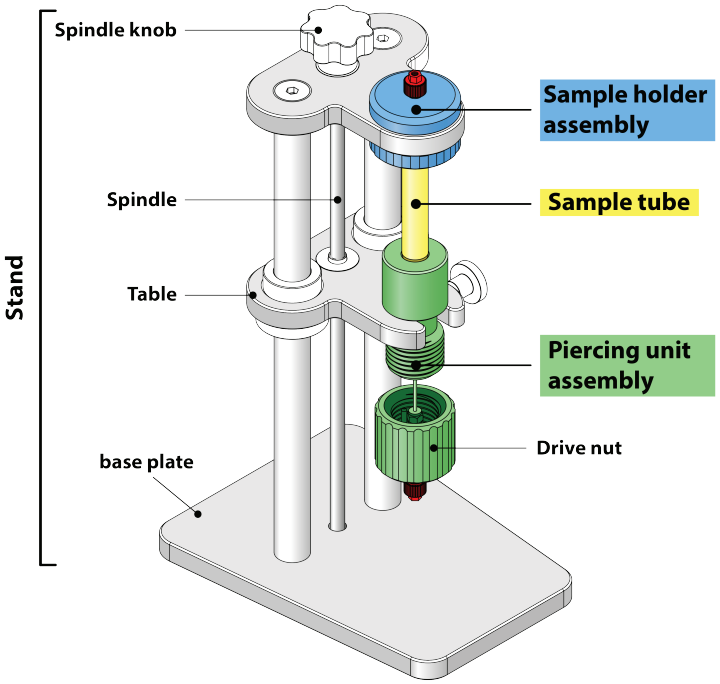
Description of parts

Naming:

- Sample holder assembly: sample holder, lock nut, large O-rings x2, small O-ring, union
- Piercing unit assembly: sample tube support, drive nut, locking nut, needle, luer adapter with O-ring, washer, needle base, small o-ring, union
- Table assembly: table (including thread), bushings x2, locking screws, knurled screw
- Stand assembly: base plate, top plate, screws x4, spindle, spindle knob, attachment of spindle

Principle of operation

The siFractor pierces the bottom of the **sample tube** to deliver a dense chase solution to displace the sample solution and to push it through the host system for analysis and fractionation. For this a polypropylene sample tube is inserted into the **sample holder assembly**, where O-rings ensure a tight fit and provide a pressure seal. Operation of the spindle knob precisely positions the robust **piercing unit assembly** underneath the sample tube and ensures its snug fit in the sample holder. Operation of the drive nut allows perforation of the sample tube with a hollow needle through which dense chase solution is delivered.



Limitations

The piercing unit is designed for open top, thinwalled polypropylene (PP) tubes with diameters of 11 mm, 13 mm, 14 mm, or 25 mm. Thickwalled PP (polyallomer) tubes can also be pierced with the system. However, due to the increase in force exerted on the piercing needle, its lifetime can be significantly reduced.

Working with the siFractor

Safety precautions

To safely operate the device, the following safety rules need to be strictly adhered to:

WARNING The device contains a needle

To avoid injuries, always retract the needle entirely into the lower casing by operating the drive nut before working on the instrument. When removing the needle from the casing, e.g. for cleaning, handle it carefully to avoid injuries.

WARNING Do not exceed the pressure limit of the device

When fractionating samples, a pressure build-up can occur. Operate the device at a **max. pressure of 1 MPa (10bar)**. If increased pressure is encountered during fractionation, stop fractionation immediately and refer to the troubleshooting section of this booklet.

WARNING Only use compatible solutions and reagents

Chemicals such as organic solvents (such as e.g. acetone) or corrosive solutions can damage the device and the sample tubes. Ensure that all solutions and reagents used for fractionation or contained within the reagent tube are compatible with the fractionator and the equipment used.
Neutral sucrose and salt solutions typically present no issues.

WARNING Additional safety measures are required when fractionating toxic, radioactive, or infectious samples

The siFractor is not intended for the fractionation of samples that contain toxic or radioactive substances, or biohazardous samples (e.g. infectious agents). For this, additional safety measures need to be set up, including, but not limited to:

- preventing of contaminations (e.g. via leaks) and release of the compounds from the system (e.g via a ruptured sample tube)
- integration of appropriate procedures for cleaning and decontamination of the system
- ensuring safe operation by appropriate personal protection and experimental procedures

WARNING Do not operate the siFractor outside of the specified conditions

The siFractor is manufactured for operation at temperature range between 4-30°C and at pressures below 1 MPa (10 bar). Use outside these conditions may damage the unit and poses a safety hazard to the user.

IMPORTANT

Always wear the proper protective equipment (gloves, laboratory coat, eye protection) when performing experiments with the siFractor

Assembly & Maintenance

IMPORTANT Do not use sharp or pointed metal tools for the removal of the O-rings, as they are easily damaged. Tip: use disposable, plastic pipet tips for O-ring removal.

Disassembly

Sample holder disassembly

Unscrew the locking nut to remove the sample holder from the stand. Carefully remove the O-rings (tip: use a disposable pipet tip to prevent damage to the O-rings).

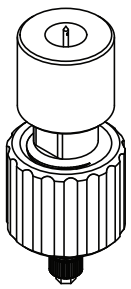
Piercing unit disassembly

WARNING: This unit contains a needle

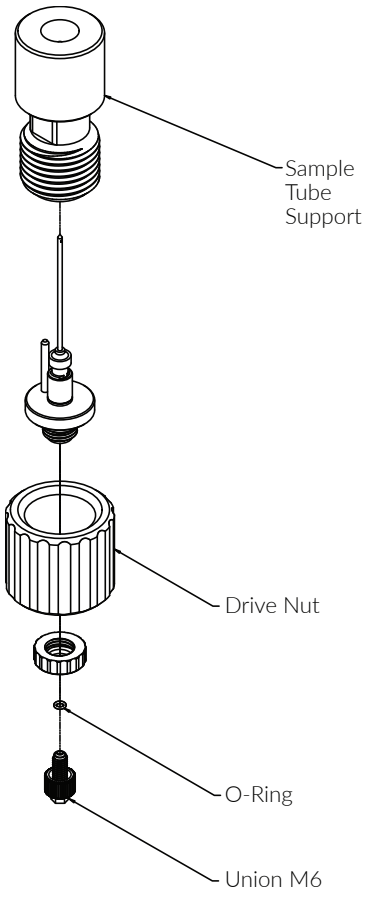
To avoid injuries during disassembly, always **retract the needle** entirely into the sample tube support by operating the drive nut. When removing the needle from its casing, work carefully to avoid injuries**.**

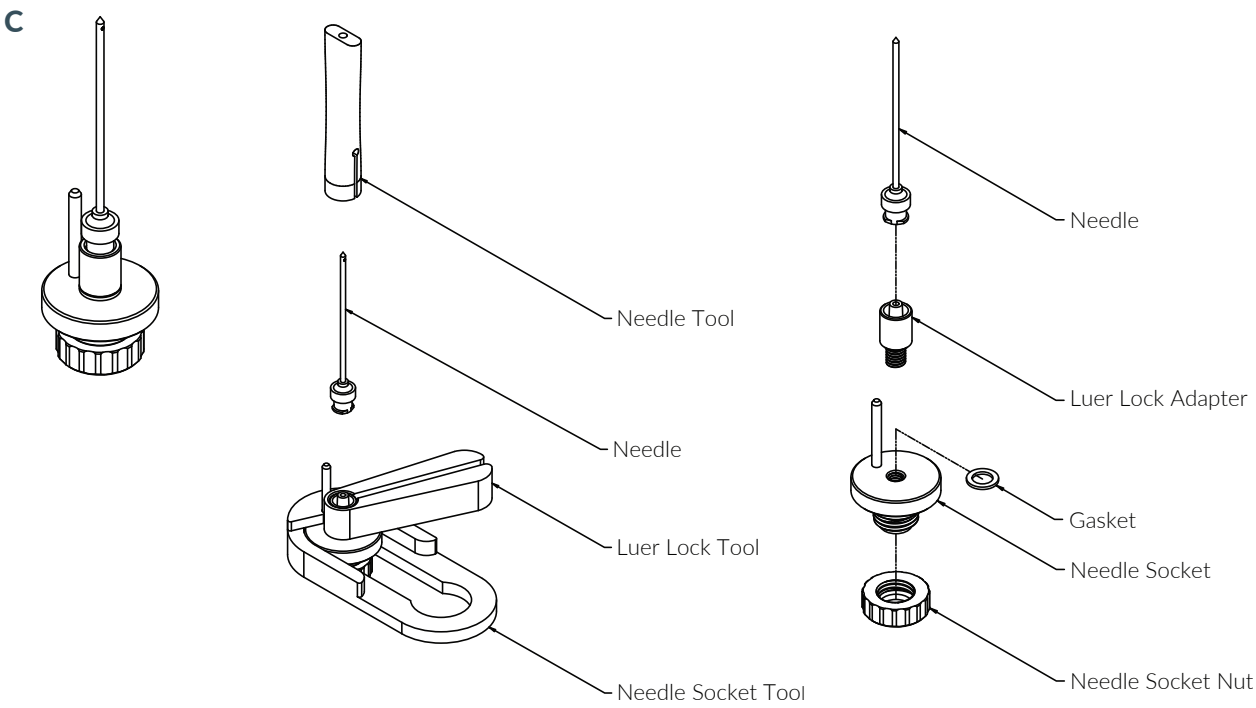
Turn the drive nut (**Fig. A**) to retract the needle fully into the sample tube support. Unscrew the knurled table screw to remove the piercing unit assembly from the table. If present, unscrew the union at the bottom and carefully remove the O-ring (tip: use a disposable pipet tip to prevent damage to the O-ring) (**Fig. B)**. Unscrew the locking nut, then the drive nut to release the needle assembly. Use the needle tool **(Fig. C)** to remove the needle from the luer adapter, then unscrew the luer adapter from the needle base.

A



B





Cleaning

The siFractor should be thoroughly cleaned after each use. For this, the sample holder assembly and the piercing unit assembly are removed from the stand. The stand itself can be wiped clean (unless the bushings of the table have been contaminated with sample or chase solution, which requires a partial disassembly). For cleaning, the piercing unit is partially disassembled (as described above) to release the needle assembly from the sample tube support.
If present, unscrew the unions and carefully remove the O-rings from the M6 threads to prevent their loss during the cleaning procedure. The O-rings in the sample holder that are used for sealing of the sample tube can remain in the unit during cleaning.
Soak all parts in a solution containing a mild detergent and carefully clean them with a brush, if required. Then thoroughly rinse the individual parts with distilled or de-ionized water to remove all traces of detergents. Air dry the disassembled parts.

IMPORTANT Never use corrosive solutions for cleaning as these might damage the unit or individual parts thereof. Instead, use solutions with mild detergents, water or ethanolic solutions for cleaning.

IMPORTANT Never use an oven to dry individual parts or the entire unit.

MPORTANT Do not autoclave the siFractor or its individual parts. This can damage the unit.

Once all parts have completely dried, reassemble the unit as described below. If required, apply small amounts of the provided grease to the O-rings. Frequently apply small amounts of PTFE lubricant to the rods of the stand to prevent corrosion and to ensure proper performance.

Assembly

Sample holder assembly

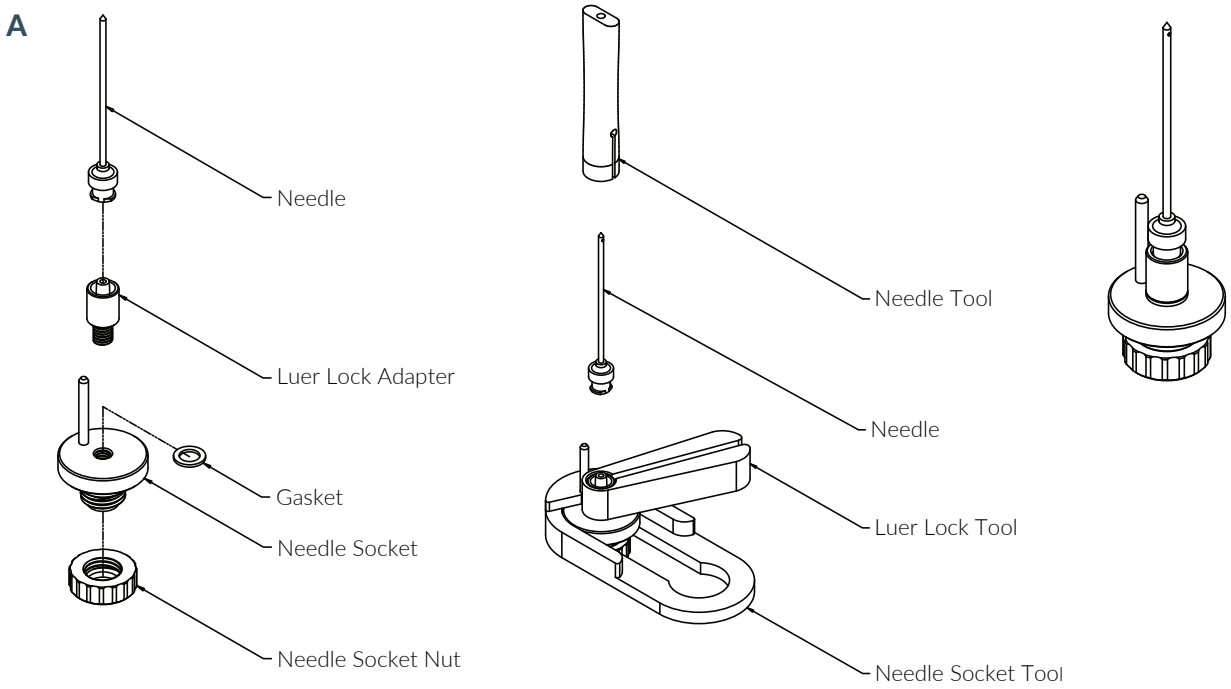
Use a little grease to lubricate the o-rings. Carefully remove excess grease with a clean paper towel, then insert the large o-rings into the groves of the sample holder and ensure proper fit. Insert the small o-ring into the M6 threaded hole and carefully push it to the bottom using a plastic tool (tip: use a disposable, plastic pipet tip). Insert the sample holder into the top plate of the stand and secure it with the lock nut. Ensure proper fit of the o-ring to prevent leaks during fractionation.

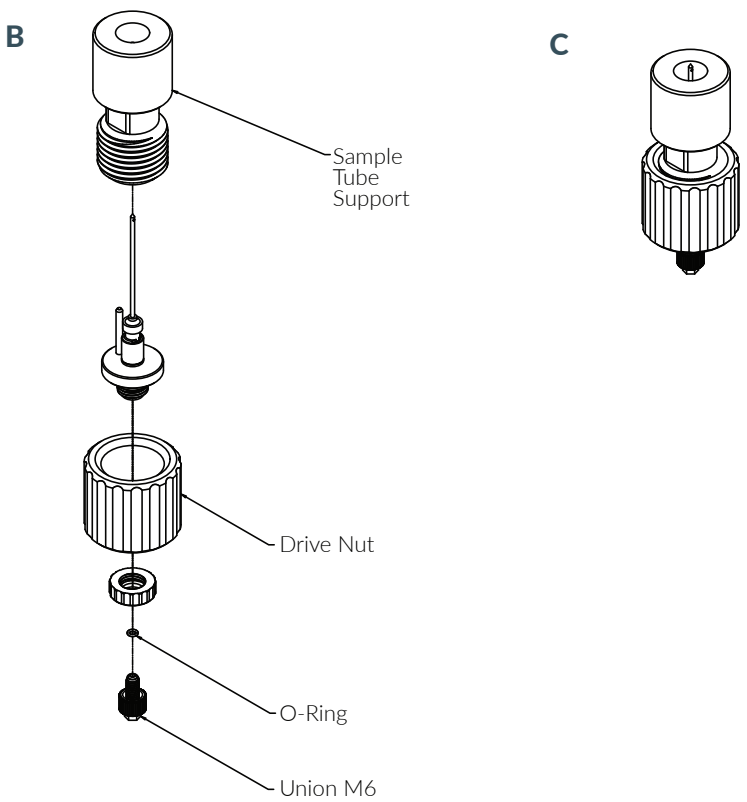
Tube piercing unit assembly

Use a little grease to lubricate the O-rings and the washer; carefully remove excess grease with a clean paper towel. Assemble the small O-ring into the groove of the luer adapter, then carefully screw the needle on top using the needle tools **(Fig. A)**. Make sure not to over-tighten the needle as this may damage the luer connection. Ensure proper fit of the o-ring to prevent leaks during fractionation

IMPORTANT Leave the needle tool on the needle during the assembly process to prevent injuries

Assemble the gasket on the luer adapter, screw it onto the needle base and tighten carefully. Insert the assembly into the sample tube support, then mount the drive nut **(Fig. B & C)**. Secure the assembly by mounting the locking nut and make sure that it is fully engaged. To minimize the risk of injuries, operate the drive nut to retract the needle into the sample tube support. Finally insert the piercing unit assembly into the notch of the table and secure it with the knurled screw.





Setup of the fractionation unit

Host system requirements

- The siFractor can be connected to a variety of host systems via the universal M6 connections. Unions are provided for 1/16" fingertight connectors used in commercially available ÄKTA systems (Cytiva). Unions for other connections need to be supplied by the user.
- As the chase solutions that are used to displace the sample are often viscous, this can result in increased pressure in the system. It is therefore recommended to use a host system that can deliver solution at a pressure of 0.5 MPa (5 bar), or higher.
- For high-precision fractionation, piston-driven pumps in the host system are recommended, as peristaltic pumps are typically less precise at delivering viscous solutions at elevated pressure.
- It is highly recommended to use a host system with a built-in 'pressure alarm' that pauses the pumps once a pre-programmed pressure limit is exceeded. Enable a pressure limit of 1 MPa (10 bar) of the host system to prevent damage to the equipment and to prevent excessive pressure in the system that might endanger the user.
- To prevent a pressure build-up during fractionation, it is recommended to equip the host system with tubing with an inner diameter of 0.75 mm or larger.
- It is recommended to use a super loop (or similar device) to deliver the chase solution. The system pump(s) can thus be operated with water, minimizing time spent on maintenance and cleaning of the host system.
- The host system should be equipped with a fraction collector and appropriate detectors
- Flow restrictors or similar devices are not recommended for the use with the siFractor

Connection to host system & initial preparations

To connect the siFractor to an FPLC or HPLC host system, M6 threads are located at the top of the sample holder and the bottom of the piercing unit assemblies. To seal the connections, small O-rings are provided. Before connecting the siFractor to the host system, make sure all parts are clean (dirt or debris can clog the tubing of the host system) and check the O-rings for wear and proper fit/positioning.
Install the siFractor in place of a column before the detector(s) of the host system. Adhere to the recommendations and instructions of the manufacturer of the host system, when connecting the siFractor.

Operating the siFractor

IMPORTANT before you start, check the host system for proper function.

IMPORTANT always enable the appropriate pressure alarm of 1 MPa (10 bar) in the host system

IMPORTANT before starting, visually inspect the siFractor for any type of damage and wear. Pay particular attention to the O-rings that engage the sample tube. A damaged siFractor must not be used.

1. Preparations

a. Enable the pressure alarm **(1MPa)**
b. Provide a sufficient supply of water or buffer to the pump(s).
c. Fill the fraction collector of the system with the appropriate tubes for collection of the sample.
d. Set the detectors of the host systems to the appropriate parameters.
e. When using a super loop or similar device for delivery of the chase solutions: fill the super loop with an appropriate amount of the chase solution. Make sure to remove all air bubbles from the super loop and connect it to the injection valve as described by the manufacturer

2. Washing

a. Insert a used but clean sample tube into the sample holder of the siFractor (tubes that have already been pierced can be used for this step). Make sure it engages the O-rings properly and that it is inserted all the way to the top.
b. Operate the spindle knob to position the piercing unit assembly below the sample tube. The sample support should completely engage the tube.
   IMPORTANT Do not overtighten the spindle! Excess pressure on the sample tube can cause it to deform resulting in a failure of the pressure seals.
c. Operate the drive nut to insert the piercing needle into the sample tube. Make sure the opening of the needle is completely inside the sample tube. This is ensured by screwing the nut to its topmost position.
d. Use the host system to deliver a steady flow of water/buffer to the sample tube. After a few moments the solution should become visible in the tube. Carefully monitor the pressure of the system – at this stage it should be very low.

e. Continue to pump water/buffer to wash the system until the monitor(s) show(s) stable reading(s). If pressure permits, washing can be performed at high flow rates (up to 10 ml/min).

IMPORTANT While in operation, check the system and all connections for leaks

f. Once the detector readings are stable, reduce the flow rate (to approx. 2 ml/min). Adjust the host system to deliver chase solution to the sample tube. Once all air bubbles have been displaced from the tubing and once chase solution becomes visible in the sample tube, pause the system and proceed to fractionation of the sample(s).

3. Fractionation

a. Cleaning

- Operate the knob to lower the table. If necessary pull on the sample tube while lowering the table to allow it to disengage from the O-rings in the sample holder assembly.
- Once the sample tube has completely disengaged from the sample holder assembly, while holding the sample tube in place turn the drive nut to retract the needle. Once the needle has been completely retracted into the sample tube support, it is safe to remove the sample tube from the system. Clean the tube piercing assembly to remove spillover of the chase solution.
- Unscrew the tubing that connects the sample holder of the siFractor to the host system on the side of the valve of the host system. Connect a syringe with the appropriate adapter to the tubing and flush it backwards first with water (or another appropriate solution) then with air. While doing so, place a clean paper towel beneath the sample holder to collect the washing solution. If necessary, remove all residual liquid from the inside of the sample holder by carefully wiping it down with a clean paper towel.

b. Fractionation/harvesting of sample

- Place a tube containing the sample carefully into the sample holder as described above (step 2a).
- Start the pump(s) to deliver chase solution at low flow rate (typically 0.5-1 ml/min) and wait a few seconds to displace air bubbles from the needle.
- Operate the drive nut to pierce the sample tube. Make sure to position the needle in a way that its opening is well within the sample tube.

  NOTE Now chase solution is delivered to displace the sample. Two separate phases should become visible in the sample tube (a colored chase solution is recommended to help visualization)
- Once the level of the chase solution has reached the tip of the needle, it is recommended to increase the flow rate to prevent trapping of air bubbles in the detector(s).

  NOTE a flow rate of approx. 5 ml/min works well for fractionation of 12 ml tubes. It can be adjusted for fractionation of smaller or larger tubes. Make sure to stay within the allowed pressure limit of the device (1 MPa) at all times during operation.

  IMPORTANT During fractionation, continuously monitor the pressure and inspect the sample tube for any deformation. Also, check the system for any leaks
- Start sample collection at will. Typically, sample collection is enabled once the sample reaches the first detector.
- Once the sample has been collected, start with fractionation of the next sample (step 3a).

4. End of fractionation

NOTE Chase solutions are often viscous and hard to remove. Make sure to remove all traces of the solution from the host system to maintain its function. This is particularly the case for highly concentrated sugar solutions that, upon drying, leave behind hard-to-remove contaminations.

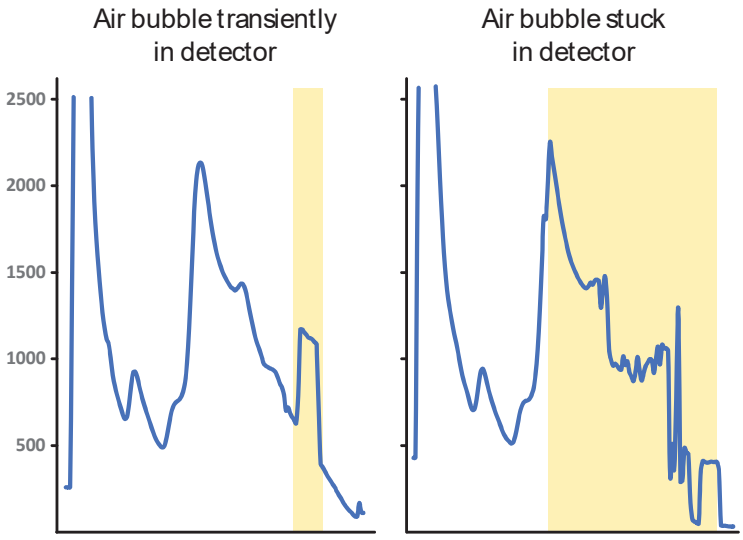
a Pause the system pump(s). Disconnect and remove the siFractor and clean it as described above

b If necessary, wash the pumps of the host system with water or an appropriate buffer, then thoroughly flush the entire system with water to remove all traces of the chase solution. Use water or another appropriate solution to thoroughly clean all connections that have been in contact with the chase solution. Disassemble equipment such as e.g. a super loop and thoroughly clean it.

Troubleshooting fractionation

Air bubble in detector

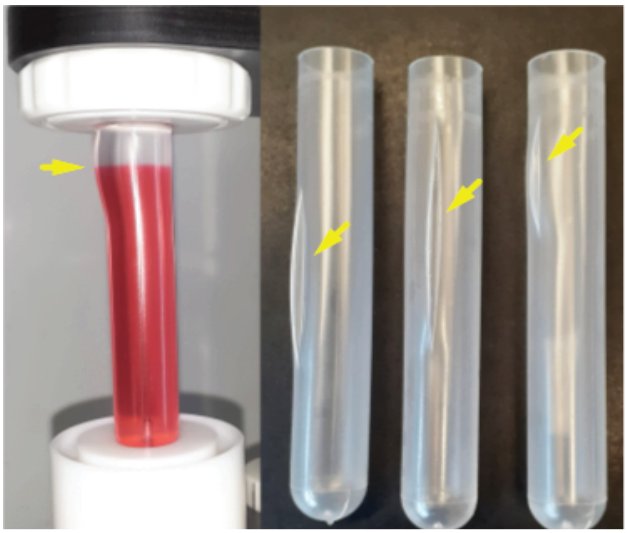
The most common problem during gradient fractionation is the formation of air bubbles which may interfere with the affect the UV reading. Small bubbles that pass the detector result in a transient increase in UV absorbance visible as small peaks in the profile. If an air bubble gets stuck in the detector, the UV-profile is severely affected. In both cases, sample collection is typically not severely compromised, and the samples can still be used for downstream applications as long as it is known in which fraction the desired samples are contained.



SOLUTION: Wash detector with warm water (optional: mild detergent solution instead of water) to remove contaminations that facilitate trapping of bubbles. Fractionate gradients at high flow rates (>2ml/min) to flush bubbles through the flow cell of the UV detector.

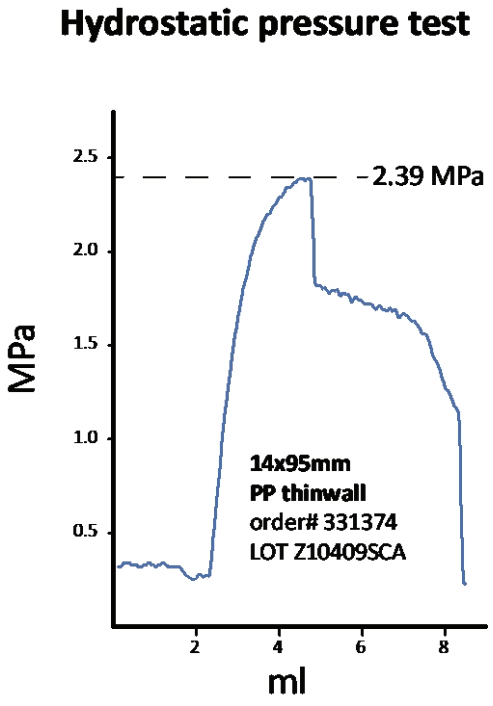
Gradient tube distortion due to pressure

In rare cases, a pressure buildup can impair fractionation. High pressure can cause the gradient tube to distort and to eventually burst. Initially, upon swelling of the tube, fractionation is impaired as the flow rate at the fraction collector does not match the flow rate of the pumps. This results in smaller volumes of the collected fractions. If the run is continued, the tube can burst and part of the gradient is lost. The pressure buildup typically occurs towards the end of fractionation when solutions with high viscosity enter the tubing. We have experimentally determined burst pressures of gradient tubes which are typically at approx. 2.4 MPa (24 bar).



SOLUTION: Remove all 'restrictions' from the flowpath that can result in a pressure buildup. For this, e.g. remove flow restrictors and/or replace tubing with a small inner diameter by tubing with a large inner diameter.

IMPORTANT: As there should be no air in the system, there will be no 'explosion' upon failure of the tube, but rather a steady and slow leak of liquid from the destroyed tube. However, if there is air in the system upon tube failure, this can result in a rapid expansion of the air bubble causing a small 'explosion' and liquid spraying from the destroyed tube. Typically, air bubbles in the system are rather small and cannot cause significant harm. Nonetheless, avoid any gas in the system, particularly downstream of the gradient. Wear safety goggles at all times during fractionation of gradients.



Supplementary Materials

Table 1. Rotor sizes

Product name	Tube diameter	Adapter label	Compatible rotors	Catalogue nb
siFractor D11	11 mm	11	SW60	eq-F001-D11
siFractor D13	13 mm	13	SW55	eq-F001-D13
siFractor D14	14 mm	14	SW41/40	eq-F001-D14
siFractor D25	25 mm	25	SW28	eq-F001-D25

Table 2. Sample holder & union M6 O-ring sizes

Product name	Inner diameter x Cross section	Type	Quantity
siFractor D11	10.5 x 2.4 mm	MVQ/VMQ	2 pcs
siFractor D13	13.0 x 2.0 mm	MVQ/VMQ	2 pcs
siFractor D14	14.0 x 2.0 mm	MVQ/VMQ	2 pcs
siFractor D25	25.0 x 3.0 mm	MVQ/VMQ	2 pcs
all siFractor models:			
siFractor O-ring	3.0 x 1.0 mm	MVQ/VMQ	2 pcs

Manual version and appropriate use

This manual and its contents are proprietary to siTOOLs Biotech GmbH and is solely intended for use by its customers for the purpose described herein. The manual and its contents shall not be used, distributed, communicated or reproduced in any way for any other purpose whatsoever without the prior written consent of siTOOLs Biotech GmbH.

The instructions within this manual should be strictly followed by qualified personnel for safe and proper use of the product(s) described herein. Failure to completely read and perform the protocol in an adequate test environment may result in damage to the product(s), injury to persons, including to users or others, and damage to other property. siTOOLs Biotech does not assume any liability arising out of the improper use of the product(s) in any form or environment.

The siFractor is developed, designed, produced and sold FOR RESEARCH PURPOSES only. No claim or representations is intended for clinical use (included, but not limited to diagnostic, prognostic, therapeutic purposes). It is rather the responsibility of the user to inspect and assure the use of the siFractor for a well-defined and specific application.

For other general terms of business and safety documentation, please refer to the siTOOLs Biotech website (www.sitoolsbiotech.com) under Resources > Other Downloads.

This manual, referred to hereby as siFractorManual_v1-0, was first created on May 11th 2022. It may be subject to future revisions. Please refer to our website for latest updates.

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