



PicoChip®

Nanospray System

Column Care & Use Manual

DOCUMENT: 000018

RELEASE DATE: January 6, 2015

Read This First

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Safety Information

Labels on PicoChip® and in this manual

All warning labels that may be displayed on either PicoChip® source or PicoChip itself are documented in this guide. Warnings and labels use international symbols.



WARNING: This symbol indicates a warning of electrical shock hazard. Read the warning and follow all precautions before performing any operation described in the manual. Failure to do so can result in serious injury.



WARNING: This symbol indicates a warning of potential burns from hot surfaces. Read the warning and follow all precautions before performing any operation described in the manual. Failure to do so can result in serious injury.



WARNING: This symbol indicates that ANSI-approved safety glasses should be worn to protect eyes in the use of indicated equipment and/or related operations. Failure to do so can result in serious injury.



WARNING: This symbol indicates that ANSI-approved rubber gloves rated for laboratory use should be worn when handling chemicals or when working with items that are impacted by handling. Failure to do so can result in serious injury or cause damage to equipment.



WARNING: This symbol indicates a warning of potential puncture injuries from sharp objects within the device. Read the warning and follow all precautions before performing any operation described.



WARNING: This symbol indicates a warning of potential injury to persons operating the instrument. Read the warning and follow all precautions before performing any operation described in the manual. Failure to do so can result in serious injury.



TIP: This symbol indicates that the information presented is beneficial to the operation of indicated equipment

Safety Considerations



WARNING: Electrospray ionization involves the use of potentially lethal high-voltage electrical current. Observe all manufacturers' safety recommendations in the use of such equipment. No equipment modifications should be made except by trained personnel using methods approved by the manufacturer in accordance with all safety requirements. Installation of equipment should be performed by qualified personnel in accordance with all applicable electrical codes.



WARNING: Never use this product with defective, damaged, or faulty equipment. Serious injury or death could result.



WARNING: Only qualified personnel should use this product. Provide a safe workplace equipped with all necessary safety equipment.

Prior to Installation

Follow all safety recommendations of the equipment manufacturer(s). All system voltages must be brought to ground potential and all high-voltage contacts disconnected from the inlet system before installation of the PicoChip® system.

Inspect all equipment carefully prior to use. Any damaged, chipped, or cracked components should not be used and must be discarded or repaired.

Handling Fused-Silica Tubing



WARNING: Handling of fused-silica or glass tubing and tips can result in serious personal injury, including eye and skin injury. Use safety glasses or goggles meeting ANSI Z87.1-1989 requirements, or the equivalent. Puncture- and chemical-resistant gloves should also be worn at all times.



WARNING: Reduce any applicable backing pressure (liquid, gas, etc.) to ambient before loosening fluidic path fittings and removing the PicoChip or transfer line from the coupling union. Prior to pressurization, make sure that components are tightened to specifications to prevent separation during use. Failure to adhere to this warning could result in projectile-like expulsion of tubing from the coupling union, which could cause serious personal injury or damage to surrounding apparatus.

The Transfer Line



WARNING: The transfer line must not be made from an electrically conductive material. It must be fabricated from an electrically insulating material such as PEEK™ or fused silica. Otherwise, the operator may be exposed to potentially lethal voltage. PicoChip transfer line complies with this safety notice.



WARNING: Do not defeat any mass spectrometer system software or hardware safety interlocks.

Specifications

Column type: Integral PicoFrit® column – column terminates in a nanospray emitter with integral frit (www.newobjective.com/products/columns/pch-2.shtml). Always apply flow and pressure in the direction toward the tip. Back flushing is not possible and will disrupt the column bed.

Bed length: 10.5 cm

Inner diameter: 50, 75, 100 or 150 µm

Tip size: 8, 10, 15 or 30 µm

Stationary phase chemistry: Variable, user specified, see label for details. Visit New Objective website for a list of in-house chemistries (www.newobjective.com/products/columns/chems.shtml).

The exact PicoChip® column specification can be found on the label located on the bottom part of each PicoChip column. Refer to the PicoChip Starter Kit User Guide (www.newobjective.com/downloads/manuals/1PCH-555-SK_Manual.pdf - Section 5 Product Specification) for label information.

Operating pressure: ≤ 5,000 psi



WARNING: The maximum operating pressure should not be exceeded. Pressures in excess of 5,000 psi may result in projectile-like expulsion of tubing from the coupling union, which could cause serious personal injury or damage to surrounding apparatus.

Solvent compatibility: PicoChip is compatible with commonly used LC solvents including water, acetonitrile, acetone, isopropanol, or methanol and commonly used modifiers (formic acid, acetic acid, ammonium acetate etc.)

Recommended flow rates: Flow rates for PicoChip vary based on the inner diameter of the column

Column ID	Recommended Flow Rate (nL/min.)
50	100 - 300
75	300 – 500
100	500 – 1,000
150	≥ 1,000

- Expected pressure depends on the resin used in column packing
- The sample load and column wash can be performed at higher than optimal flow rate as long as the pressure of the column is kept under 5,000 psi
- PicoChip columns with 100 or 150 µm ID can be used at flow rates up to 1 µL/min. Higher flow rates are possible, but not generally recommended as stability may be compromised. Empirical testing of mobile phase composition and applied voltage is the best solution if you want to operate at higher flow rates.

Recommended voltage settings: For optimal results, use the following recommended flow rates and corresponding operating voltage ranges for PicoChip®.

Mobile Phase Composition (%B)	Flow rate (nL/min.)	Recommended Spray Voltage (kV)
2	200	1.8 - 2.1
2	300	1.8 - 2.3
2	500	2.3 - 2.7
25	200	1.6 - 1.9
25	300	1.6 - 2.0
25	500	1.8 - 2.1

PicoChip Column Equilibration

Equilibration Protocol

PicoChip columns are packed and shipped in methanol and should be equilibrated prior to use in sample injections.



TIP: Turn the voltage on during the column equilibration to prevent droplet formation on the tip of the column.

- Position the PicoChip on the PicoChip source and connect the column to the LC pump
- Expose the tip and equilibrate the column at the same mobile phase conditions as used for sample loading. The column should be equilibrated with 10-20 column volumes of solvent. The minimum equilibration volume (10x the column volume) is listed in the following table:

PicoChip Column ID (µm)	Minimum Equilibration Volume (µL)
50	2.06
75	4.63
100	8.25
150	18.55

- Blank gradient with a blank injection is an efficient way to equilibrate the column.

PicoChip Column Validation and Conditioning

System validation is an important part of obtaining reproducible results. PicoChip columns are rigorously inspected and tested before use. Given nanoLC-MS system complexity, it is recommended that each new column be conditioned and system performance verified with a defined standard prior to injecting valuable or precious samples.

PicoChip® column conditioning and validation protocol

- Prior to peptide analysis, the column can be initially conditioned with one high concentration injection, or by multiple low concentration injections of standard protein digest. New Objective uses one injection of 10 pmol of BSA or enolase digest on the column for column conditioning.
- Once conditioned, daily evaluation of system performance using a PicoChip® column together with a commercially available peptide mixture (e.g. PicoSure standard from New Objective - www.newobjective.com/products/columns/picosure.shtml) or peptide digest is recommended.



TIP: Conditioned columns perform consistently so the retention time, peak shape and peak intensity should be reproducible.

Preventative Maintenance

- Store the column in the original unopened packaging until ready to use
- The use of bottled and filtered (0.45 µm or better) HPLC grade water is recommended. If possible avoid the use of DI water from in house filtering stations. Small carbon particles escaping from the filter cartridges may accumulate in the column and increase the pressure of the column (refer to Troubleshooting Clogs section on our website (www.newobjective.com/support/tipstricks.shtml)).
- Flush the system for several minutes at high flow rate prior to connecting a new column
- Use preventative plumbing (e.g. inline filters) to prevent particle accumulation behind the bed of the column
- Periodically wash the tip of the column with few drops of methanol to minimize sample accumulation around the tip of the column



WARNING: The fused-silica tip of the PicoChip column is very fragile. Contact between any surfaces, including skin, will result in damage to the spray tip. Exposing the tip when the PicoChip is not mounted on the source is not recommended. Use of ANSI-approved safety gloves is recommended when handling PicoChip columns or any fused-silica tubing.

Disconnecting and Storing PicoChip Columns

Short term storage (hours or overnight)

- Run minimally one blank injection at the end of the sample sequence
- Stop the flow
- Turn voltage off
- Spray a few drops of methanol on the tip to clean away any accumulated debris
- Lift the PicoChip column from the source to retract the tip back into the PicoChip column body



TIP: Retracting the tip back into the PicoChip body will protect the tip from damage due to accidental contact, but also protects the tip from environmental particulate accumulation (ambient).

- The PicoChip® can be left on the source or set side on top of the LC pump or mass spectrometer. It is not necessary to disconnect the column from the LC pump if the run is interrupted only for few hours.

Long term storage (days to weeks)

- Run minimally one blank injection at the end of the sample sequence
- If possible, wash the column with 100% methanol or 100% acetonitrile with no modifier. If pure solvents are not available, wash the column with 100% organic phase used for the run.
- Stop the flow
- Turn voltage off
- Wait until the pressure goes to zero and the flow completely stops, then disconnect the column from the LC pump
- Wash the tip of the column with few droplets of methanol
- Remove the PicoChip from the source by lifting up on the PicoChip
- Coil fused-silica tubing pig tail (not applicable for nanoViper PicoChip columns), insert the column back into the original plastic box and store the column at the room conditions until the next use.