

PicoChip Nanospray Starter Kit

1 Introduction

New Objective has developed an integrated system for nanobore LC-MS that alleviates the need for specialized expertise in nano-scale separations. The novel design of the PicoChip combines the functionality of the nanospray emitter, nanobore separation column, high-voltage contact, and autosampler transfer line into a single consumable device. The comfortable design of the consumable package makes it exceptionally easy to handle and eliminates the risk of emitter or column breakage. A new high-voltage contact with every column change means consistent and stable spray ionization. Pre-assembly and testing of the assembled device in a production setting ensures results in the customer's lab.

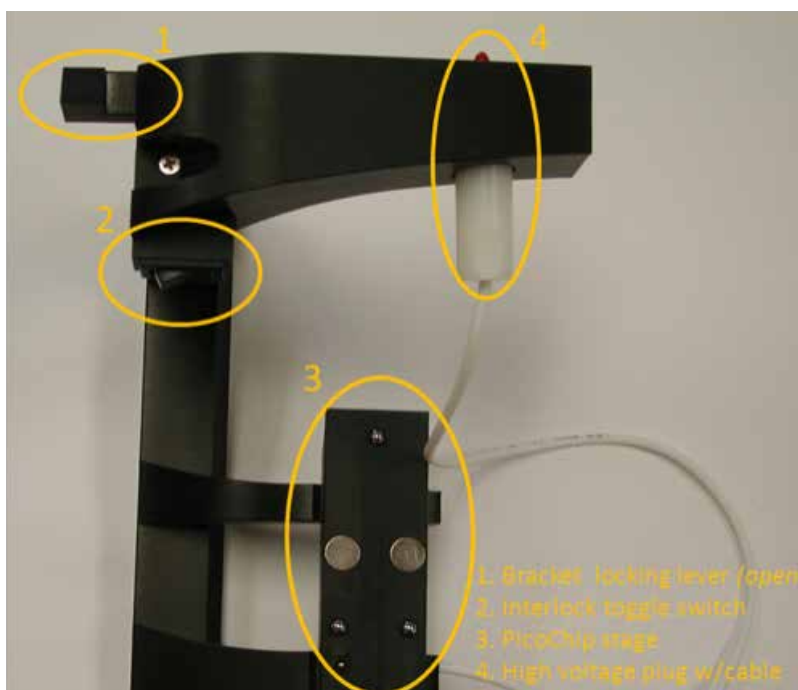
The PicoChip Starter Kit includes everything you need to get started in the lab using PicoChip technology; the PicoChip source, PicoChip columns and the PicoChip Alignment Chip. These instructions will help you install the source and PicoChip so you can be collecting data in a matter of minutes!

For more information please visit our website www.newobjective.com.

2 PicoChip Starter Kit Components

- (1) PicoChip source compatible with Thermo LTQ, Orbitrap (including Q Exactive) and TSQ instruments
- (2) PCH7515-105H002 PicoChip columns with voltage keys:
75 μm ID x 15 μm tip ID x 105 mm ProteoPep II 5 μm 300 \AA C18
- (1) PicoChip alignment chip

2.1 PicoChip Source



2.2 PicoChip Alignment Chip

2.2.1 PicoChip Alignment Chip Overview

The PicoChip Alignment Chip is an alignment tool included with the PicoChip Starter Kit. The purpose of the Alignment Chip is to validate the positioning and alignment of PicoChip columns on the PicoChip source stage relative to the MS inlet.



2.2.2 PicoChip Alignment Chip Shipping Conditions

The PicoChip Alignment Chip will ship pre-installed on the PicoChip stage. Please remove the Alignment Chip from the stage before installing the source onto your instrument.



2.3 PicoChip



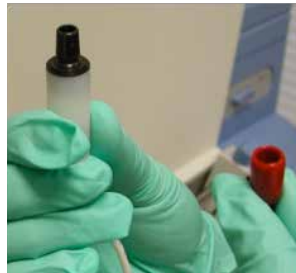
3 Getting Started with PicoChip

3.1 Install PicoChip source

With the bracket locking lever open, install the PicoChip source onto the instrument.

Close the bracket locking lever to secure the source onto the instrument.

Remove the protective cover from the high voltage plug and install the high voltage plug into the receptacle.



3.2 Check Alignment with the PicoChip Alignment Chip

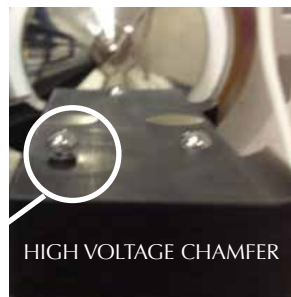
Visually validate PicoChip alignment with MS inlet using PicoChip Alignment Chip.



3.3 Installing PicoChip onto PicoChip Source

Place PicoChip onto magnetic stage of PicoChip source. The magnets on the chip provide automatic and precise alignment with the magnets on the magnetic stage.

The voltage contact on the stage has a chamfer (or beveled edge). The chamfer functions as a safety feature preventing users from making direct contact with the high voltage path. Ensure the voltage pin on the PicoChip has aligned properly with the voltage contact on the source stage.



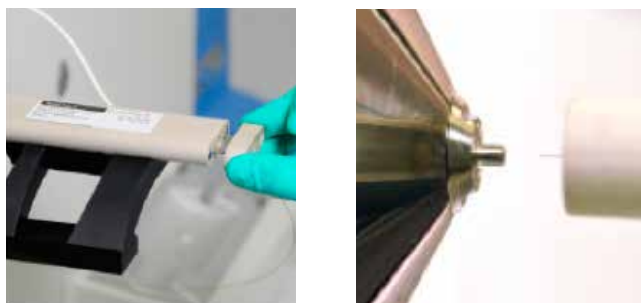
3.4 Connecting the Transfer Line

Connect the PicoChip transfer line to your LC system.



3.5 Voltage Key Engagement

Insert engagement key to expose the tip and activate the internal voltage connection.

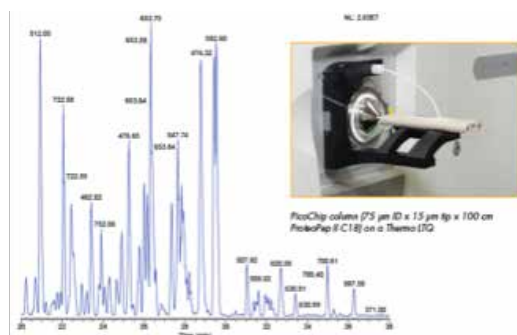


3.6 Engage Source Interlock

Close the interlock switch on the source. In LTQ Tune the software read back will change from 'Source is Open' to 'Instrument is in Standby' once the interlock switch has been closed.



3.7 Collect data!



4 Recommended Operating Conditions

For optimal results, use the following recommended flow rates and corresponding operating voltage ranges for PicoChip columns with 75 μm ID and 15 μm tip ID formats.

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

5 Product Specifications

5.1 PicoChip Alignment Chip



The PicoChip Alignment Chip can be installed onto the PicoChip stage magnetically with automatic positional alignment, identical to PicoChips. Use the Alignment Chip for visual validation of your emitter's position relative to the MS inlet. The tip of the Alignment Chip should align concentrically with the inlet on the MS. Please contact New Objective if any deviations in alignment are observed.

5.2 PicoChip

The following components comprise the PicoChip assembly:

PicoFrit column

Packed emitter manufactured from 360 μm OD fused silica tubing, 105 mm in length. The exact tubing ID, tip ID, packed bed length dimensions and stationary phase will be variable and as specified by the customer when ordering PicoChip. Please refer to the PicoChip product label for column dimensions and details (located on the bottom of the PicoChip).

High Voltage Liquid Junction

Valco 360 μm Nanovolume union, HPLC grade stainless steel (~7 nl swept volume) fitted with two PEEK nut/ferrule fittings

Transfer Line Tubing

PreCut fused silica tubing, 360 μm OD x 25 μm ID x 50 cm length. The inlet to the PicoChip column is precut for a clean square connection to your LC system, but can be manually cleaved to a shorter length to accommodate your specific instrument configuration.

PicoChip Body

Manufactured from chemically inert and heat resistant nylon, the PicoChip body houses all of the components while protecting the emitter from damage until data is ready to be acquired.

High Voltage Pin

Integral gold high voltage contact pin for applying voltage to the high voltage liquid junction inside the chip body.

High Voltage Engagement Key

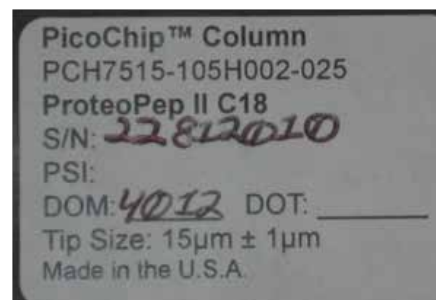
A multifunctional key used to engage the HV contact internally while exposing the tip for data collection. The engagement key is made of the same chemically inert nylon as the PicoChip body.

PicoChip Label (located on the bottom of the PicoChip)

The PicoChip label provides key information about the PicoChip.

- PCH7515-105H002-025 – PicoChip part number
 - PCH - PicoChip
 - 75 - 75 μm ID, tubing ID of column
 - 15 - 15 μm ID tip size
 - 105 -105 mm bed length
 - H002 - media code for ProteoPep II 5 μm 300 Å C18
 - 025 - 25 μm ID, tubing ID of connecting tubing
- ProteoPep™ II C18 – stationary phase
- S/N: serial number of the PicoFrit column
- PSI: PicoChip pressure recorded at 1 $\mu\text{L}/\text{min}$ 100% methanol
- DOM – date of manufacture of the PicoChip
- DOT – date PicoChip was pressure tested

Tip Size: 15 \pm 1 μm – tip measurement specifications



6 Trademarks

The following trademarks may be referenced in this instruction manual:

Teflon is a registered trademark of E.I. du Pont de Nemours and Co.; LTQ, Orbitrap, Q Exactive, TSQ, Quantum, and Xcalibur are trademarks or registered trademarks of Thermo Scientific; MicroTight and SealTight are trademarks or registered trademarks of Upchurch Scientific, Inc.; PEEK and PEEKsil are trademarks of Victrex plc; PicoChip, PicoTip, PicoFrit, SilicaTip, TaperTip, and are trademarks or registered trademarks of New Objective, Inc. All other trademarks are the properties of their respective companies.

7 Limited Warranty

DISCLAIMER

Technical information contained in this publication is for reference purposes only and is subject to change without notice. The information is believed to be reliable and accurate; however, nothing set forth herein constitutes a warranty of any kind or nature. Given the variety of experimental conditions, New Objective, Inc., cannot guarantee performance at a given flow rate; the best guide to tip selection and operation is empirical testing.

NOTICE: The user is solely responsible for complying with any patent(s) pertaining to applications or methods using the products described or mentioned in this manual.

New Objective, Inc., warrants this Product (PicoChip™ source system) to be free of defects in materials and workmanship for a period of one (1) year from the date of shipment. New Objective, Inc., warrants the accompanying PicoChip columns to be free of defects in materials and workmanship for a period of ninety (90) days from the date of shipment. Any item believed to be defective within the meaning of the foregoing sentence shall be returned to New Objective, Inc., and, if found by us to be defective, shall be repaired or replaced with conforming Product of like kind. Please note that a Return Authorization Number will be required. New Objective, Inc., will pay return freight on unsatisfactory items. New Objective, Inc., shall have no other liability or obligation with respect to goods alleged to be defective. The foregoing shall constitute the sole and exclusive remedy, and New Objective, Inc.'s total liability for any and all losses and damages arising out of any cause whatsoever (whether such cause be based in contract, negligence, strict liability, other tort, or otherwise) shall in no event exceed the purchase price of the Product(s) in respect of which the cause rose. New Objective, Inc., disclaims, and shall not be liable, in any event, for loss of profits, consequential or incidental damages, or punitive or exemplary damages in connection with the Product furnished hereunder.

The foregoing limited warranty (i) shall be void as to any item of Product which is in any material respect altered by the user, and (ii) does not cover misuse of the Product (for example, but not limited to, dropping or other mishandling of any components of PicoChip source or column, improper trimming of fused-silica tubing, damage caused by application of or exposure to excessive temperature, pressure, or voltage or failure by reason of clogging).

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Use and Conditioning of PicoChip Columns

- o Fritted on one end only - the outlet
- o Frit Loss
 - Tip contains frit
 - Resin held in column behind frit
 - Broken tips (contains frit)
 - resin loss under pressure
 - Removal due to pressure
 - resin loss under pressure
- o Shifting of packed bed during shipping (no frit on inlet)
 - Need to re-pressurize column prior to cleaving length
 - Particularly apparent for AQUASIL (media code H053)

Pressurizing a PicoChip Column

- o Plumb distal end of column into HPLC system (through sleeves, fittings, etc.)
- o Cleave ~5 cm fused silica tubing from distal end of 50 cm length column using recommended cleaving tools
 - Diamond Scribe
 - Shortix
- o Connect column to flow outlet of HPLC system
- o Start HPLC pump
 - Composition = 98% Mobile Phase A, 2% Mobile Phase B
 - Flow Rate = 200 nl/min – 1 ul/min
 - dictated by pressure limit of HPLC pump
- o Pressurize for 10 minutes
- o Record pressure, flow rate and composition
- o Turn flow off
- o Allow system pressure to decrease
- o Disconnect column from LC system
- o a sudden change in pressure will further disrupt the packed bed
- o Cleave column to operating length
 - e.g. 10.5 cm to 15 cm for 10 cm column bed

Flow Rate

- o 75 µm ID column flow rate range
 - 0.2 µl/min – 0.5 µl/min.
- o Optimum flow rate
 - 0.3 µl/min.
- o Expected pressure dependent on several variables
 - particle size of the material
 - bed length of the column

Column Equilibration

- o New Objective columns shipped in 100% MeOH
- o Equilibrate columns with the same mobile phases used for experimental conditions
 - e.g. Mobile Phase A = 0.1% Formic Acid in Water
 - e.g. Mobile Phase B = 0.1% Formic Acid in Acetonitrile
- o Blank gradient simplest way to equilibrate column
 - no sample injection
 - injection of a sample of initial mobile phase conditions
 - 98% Mobile Phase A, 2% Mobile Phase B

Column Conditioning

- o Evaluate column performance using a well characterized standard
 - commercially available peptide mixture
 - commercially available peptide digest
- o Evaluate column performance when the column is fully conditioned
- o Conditioned columns perform consistently
 - Peak shape
 - Peak Height
 - RT
- o Total number of injections required for conditioning
 - 2-10 injections

Available Chromatography Media

As of January 2013

Order Code	Chemistry Name	Type, Particle, Pore Size	Manufacturer
H001	ProteoPep™	C18, 5 µm, 300 Å	New Objective
H004	ProteoPep™ II	C4, 5 µm, 300 Å	New Objective
H005	ProteoPep™ II	C8, 5 µm, 300 Å	New Objective
H002	ProteoPep™ II	C18, 5 µm, 300 Å	New Objective
H003	ProteoPep™ III	C18, 1.8 µm, 80 Å	New Objective
H020	Strong Cation Exchange	SCX, 5 µm, 85 Å	New Objective
H035	HALO® C18	2.7 µm, 90 Å	AMT, Inc.
H036	HALO® C8	2.7 µm, 90 Å	AMT, Inc.
H037	HALO® RP	2.7 µm, 90 Å	AMT, Inc.
H038	HALO® HILIC	2.7 µm, 90 Å	AMT, Inc.
H039	HALO® Peptide ES	C18, 2.7µm, 160 Å	AMT, Inc.
NEW H080	ProntoSIL	C18 AQ, 3µm, 200 Å	Bischoff
H050	BioBasic®	C4, 5µm, 300Å	Thermo Scientific
H051	BioBasic®	C8, 5µm, 300Å	Thermo Scientific
H052	BioBasic®	C18, 5µm, 300Å	Thermo Scientific
H053	AQUASIL	C18, 5µm, 100Å	Thermo Scientific
H054	Betasil® C18	5µm, 100Å	Thermo Scientific
H060	BetaBasic®	C8, 5µm, 150Å	Thermo Scientific
H061	BetaBasic®	C18, 5µm, 150Å	Thermo Scientific
H062	Hypercarb	C100, 5µm, 250Å	Thermo Scientific
H063	Hypercarb	C100, 7µm, 250Å	Thermo Scientific
H070	Hypersil GOLD® C18	5µm, 175Å	Thermo Scientific
H071	Hypersil GOLD® C18	1.9µm, 175Å	Thermo Scientific
H072	Hypersil GOLD PFP™	PFP, 5µm, 175Å	Thermo Scientific
H076	Hypersil GOLD PFP™	PFP, 3µm, 175Å	Thermo Scientific
H075	Hypersil GOLD PFP™	PFP, 1.9µm, 175Å	Thermo Scientific
H073	Hypersil GOLD aQ™	C18, 1.9µm, 175Å	Thermo Scientific
H074	Hypersil GOLD aQ™	C18, 5µm, 175Å	Thermo Scientific
H100	XBridge™	C8, 3.5µm, 135Å	Waters
H101	XBridge™	C8, 5 µm, 135Å	Waters
H102	XBridge™	C18, 3.5µm 135Å	Waters
H103	XBridge™	C18, 5 µm, 135Å	Waters
H110	XTerra® C18	5 µm, 125Å	Waters
H200	Inertsil® ODS-3™	C18, 3µm, 100Å	GL Sciences
H201	Inertsil® ODS-3™	C18, 5µm, 100Å	GL Sciences
H202	Inertsil® ODS-4™	C18, 2µm, 100Å	GL Sciences
H203	Inertsil® ODS-4™	C18, 3µm, 100Å	GL Sciences
H204	Inertsil® ODS-4™	C18, 5 µm, 100 Å	GL Sciences
H210	Titansphere®	TiO2, 5µm	GL Sciences
H252	MAGIC AQ®	C18, 5µm, 200 Å	Michrom
H253	MAGIC AQ®	C18, 3µm, 200 Å	Michrom
H401	Monitor®	C18, 3 µm, 100 Å	Orochem Technologies
H402	Monitor®	C18, 5 µm, 100 Å	Orochem Technologies
H403	Gazelle™	C18, 1.7 µm, 100 Å	Orochem Technologies
H301	POROS-R2	C18-AQ, 20 µm, 2000 Å	Life Technologies/ABI

Available Chromatography Media

As of January 2013

H302	POROS-R2	C18-AQ, 10 μm , 2000 Å	Life Technologies/ABI
H354	Reprosil-PUR	C18-AQ, 3 μm , 120 Å	Dr. Maisch
H355	Reprosil-PUR	C18-AQ, 3 μm , 200 Å	Dr. Maisch
H501	PLRP-S	5 μm , 4000Å	Varian
H502	PLRP-S	5 μm , 1000Å	Varian