

## AbsoluteIDQ® Bile Acids kit requirements

This document specifies additional lab equipment and chemicals that are required but not included with the kit. Before starting with the kit, ensure that all required items are available.

### LC-MS system

Agilent	SCIEX	Thermo Scientific	Waters
<b>Mass spectrometer</b>			
<b>Agilent 6495C</b> Triple Quadrupole LC/MS System	<b>SCIEX 5500, 5500+, 6500 or 6500+</b> series (QTRAP or Triple Quad) with TurboV™ ion source	<b>Thermo Scientific TSQ Vantage</b> with HESI-II ion source	<b>Waters Xevo TQ-S or TQ-XS</b> with ESI source
<b>LC and autosampler</b>			
<ul style="list-style-type: none"> <li>– Agilent 1290 Infinity I/II UHPLC system with column oven.</li> <li><b>NanoLC and microLC are not supported!</b></li> <li>– 1290 Infinity II inline filters, 0.3 µm (2 pcs., before and after injection valve, Agilent part no. 5067-6189)</li> <li>– 96-well plate autosampler with temperature control (10 °C)</li> <li>– Injection volume: 10 µL</li> </ul>	<ul style="list-style-type: none"> <li>– Standard flow HPLC or UHPLC system with column oven</li> <li><b>NanoLC and microLC are not supported!</b></li> <li>– 96-well plate autosampler with temperature control (10 °C)</li> <li>– Injection volume range: up to 20 µL</li> <li>– Special sample rack for deep-well plates required for the following autosamplers:               <ul style="list-style-type: none"> <li>– Shimadzu: part no. 228-37546-92 (Deep-well MTP Rack, DWP-96)</li> <li>– UltiMate 3000: Thermo part no. 6820.4083 (30 – 36 mm height)</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>– Waters ACQUITY UPLC® system with sample manager, solvent manager, and column oven:               <ul style="list-style-type: none"> <li>– Classic (loop-based)</li> <li>– I-Class (FTN)</li> <li>– H-Class (FTN)</li> <li>– Injection volume: 10 µL</li> </ul> </li> </ul>	
<b>Tubing</b>			
<ul style="list-style-type: none"> <li>– Recommended between injector and column system: steel capillary</li> <li>– Recommended between column system and mass spectrometer: red PEEK Tubing 1/16" OD x 0.005" ID</li> </ul>			
<b>Finger tight capillary ferrules</b>			
<ul style="list-style-type: none"> <li>– Sure-Lok Nut, High Pressure, PEEK, for 1/16" OD Tubing, 10-32 Thread, 12,000 psi (Phenomenex AQ0-8503)</li> <li>– Sure-Lok Fitting Tightening Tool Aluminum (Phenomenex AQ0-8530)</li> </ul>			

## LC-MS instrument status

Condition
<ul style="list-style-type: none"> <li>– Serviced, calibrated and tuned instruments according to manufacturer’s recommendations on a regular basis</li> <li>– LC-MS system must be free of ion-pair reagent</li> </ul>

## Analytical column

HPLC and UHPLC	
Analytical column	AbsoluteIDQ® Bile Acids kit column: biocrates product number 9120052120868
Precolumn	AbsoluteIDQ® Bile Acids kit precolumn: biocrates product number 9120052120875
Precolumn holder	AbsoluteIDQ® Bile Acids kit setup box: biocrates product number 9120052120882

## Software

Agilent	SCIEX	Thermo Scientific	Waters
MS software			
MassHunter version 10.1 or later	Analyst® version 1.5.2 or later	Xcalibur™ version 2.2 or later	MassLynx® version 4.1 or later
Data processing computer			
<ul style="list-style-type: none"> <li>– Oracle® database:               <ul style="list-style-type: none"> <li>– <b>Option 1 (recommended):</b> full commercial version, updates and support provided by Oracle. Expert required. Compatible versions: 11g, 12c, 18c, 21c</li> <li>– <b>Option 2:</b> Oracle Database Express Edition (Oracle XE), free of charge, provided by biocrates. No updates and support provided by Oracle. Compatible versions: 11g, 12c, 18c, 21c</li> </ul> </li> <li>– RAM for Oracle XE: Computer with up to 32 GB RAM. Contact biocrates support if a computer with more than 32 GB RAM is used.</li> <li>– MetIDQ™ software (provided by biocrates)</li> <li>– Windows 7 or later, 64-bit architecture</li> <li>– Microsoft .NET Framework 4.5.2 or later</li> <li>– Microsoft Visual C++ redistributables (32 &amp; 64 bit each): 2005, 2008, 2010, 2012, and 2013</li> </ul>			

## Solvents and chemicals

Solvents and chemicals	Purity
Acetonitrile, isopropanol, methanol, water	LC-MS grade
Formic acid	LC-MS grade (e.g. Honeywell Fluka™ 56302-50ML) <b>Fresh! New bottle or opened within past 6 months</b>
Ammonium acetate	LC-MS grade
Phosphate buffered saline (PBS)	p.a. grade (e.g. Sigma-Aldrich P4417)

## Laboratory equipment

Laboratory equipment		
	Nitrogen evaporator for 96-well plates	Pressure manifold for 96-well plates
Nitrogen evaporator* <u>or</u> pressure manifold* > <b>Requires nitrogen supply</b> > <b>Must be in a fume hood</b>	<b>Examples:</b> – Porvair blowdown evaporator MiniVap® – Techne (FSC496D) – Sample concentrator from BenchTop Lab Systems (96 samples, BT1604) – VLM evaporators – Organomation MICROVAP microplate evaporator – Biotage TurboVap® 96	<b>Examples:</b> – Waters positive pressure-96 processor (186006961) – Biotage® PRESSURE+ 96 manifold (PPM-96) – CEREX® system 96 processor (288-0001) – Agilent positive pressure manifold 96 processor (PPM-96) – TECAN Resolvex® M10 96
Centrifuge	Must be able to centrifuge 96-well plates of 5 cm height at 500 x g.	Not required when a pressure manifold is used.
Shaker	Any model with adjustable speed (450 – 1200 rpm) including a tray for plates and vials. Recommended: Eppendorf MixMate® or ThermoMixer®	
Pipettes	– Repeater, e.g. Eppendorf Multipette® E3 (with 1.0 and 10 mL tips) or similar electronic model – Single channel: volume range 10 µL – 1000 µL	
Vortexer	Any model	
Balance	Accuracy < 1 mg	
Solvent bottles	50 – 1000 mL	

\* See document “Technical guide-Nitrogen evaporators and pressure manifolds” for details.