

## 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><br>Triple Quadrupole LC/MS System   | <b>SCIEX 4000, 4500, 5500, 5500+, 6500 or 6500+</b> series 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>– <b>Serviced, calibrated and tuned instruments</b> according to manufacturer’s recommendations on a regular basis</li> <li>– LC-MS system must be <b>free of ion-pair reagent</b></li> </ul> |  |

## Analytical column (all items included in the starter package!)

| HPLC and UHPLC    |   |
|-------------------|---|
| Analytical column | <b>AbsoluteIDQ® Bile Acids kit column:</b><br>biocrates product number 9120052120868                            |
| Precolumn         | <b>AbsoluteIDQ® Bile Acids kit precolumn:</b><br>biocrates product number 9120052120875                         |
| Precolumn holder  | <b>Included in the AbsoluteIDQ® Bile Acids kit starter package:</b><br>biocrates product number 9120052121940.9 |

## Software

| Agilent   | SCIEX                              | Thermo Scientific                 | Waters                            |
|---|------------------------------------|-----------------------------------|-----------------------------------|
| MS software                                     |                                    |                                   |                                   |
| MassHunter<br>up to version 10.1<br>(not later) | Analyst®<br>version 1.6.2 or later | Xcalibur™<br>version 2.2 or later | MassLynx®<br>version 4.1 or later |

### biocrates WebIDQ workflow manager system requirements

#### WebIDQ cloud subscription – biocrates part number 21629.1

- Web browser: Edge or Chrome (latest version)
- 8 GB RAM
- 4 cores (Intel 8<sup>th</sup> generation, AMD 5000 series or comparable)

#### WebIDQ on-premises subscription – biocrates part number 21636.1

- Web browser: Edge or Chrome (latest version)
- 8 GB RAM
- 4 cores (Intel 8<sup>th</sup> generation, AMD 5000 series or comparable)
- WebIDQ server
  - Windows 10 or later, 64-bit architecture
  - 16 GB RAM
  - 6 cores (vCPU)
- Database options
  - Recommended: PostgreSQL version 12 or later
  - Oracle® version 21c or later
    - Option 1: full commercial version, updates and support provided by Oracle. Specialist required.
    - Option 2: Oracle Database Express Edition (Oracle XE). Free of charge. 12 GB maximum amount of user data. No updates and support provided by Oracle or biocrates. Computer with up to 32 GB RAM maximum.

## 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)<br><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*<br><u>or</u><br>pressure manifold*<br>> <b>Requires nitrogen supply</b><br>> <b>Must be in a fume hood</b> | <b>Examples</b><br>– Porvair blowdown evaporator MiniVap®<br>– Techne (FSC496D)<br>– Sample concentrator from BenchTop Lab Systems (96 samples, BT1604)<br>– VLM evaporators<br>– Organomation MICROVAP microplate evaporator<br>– Biotage TurboVap® 96 | <b>Examples</b><br>– Waters positive pressure-96 processor (186006961)<br>– Biotage® PRESSURE+ 96 manifold (PPM-96)<br>– CEREX® system 96 processor (288-0001)<br>– Agilent positive pressure manifold 96 processor (PPM-96)<br>– 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<br>– 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.