

AbsoluteIDQ® p180 kit requirements

LC-MS system

Agilent	SCIEX	Thermo Scientific	Waters
Mass spectrometer			
6470 Triple Quadrupole	SCIEX 4000, 4500, 5500, 6500 or 6500+ series (QTRAP or Triple Quad) with TurboV™ ion source	Thermo Scientific TSQ Vantage with HESI-II ion source	Waters Xevo TQ-XS, TQ-S or TQ-S micro with ESI source
LC and autosampler			
<ul style="list-style-type: none"> – Agilent 1290 Infinity I/II UHPLC system with column oven. NanoLC and microLC are not supported! – Agilent Infinity II: Inline Filter Kit <u>required</u> (p/n 5067-6189) – 96-well plate autosampler with temperature control (10 °C) – Injection volume range: 2 – 20 µL 	<ul style="list-style-type: none"> – Standard flow HPLC or UHPLC system with column oven. NanoLC and microLC are not supported! – 96-well plate autosampler with temperature control (10 °C) – Injection volume range: 2 – 20 µL – Special sample rack for deep-well plates required for the following autosamplers: <ul style="list-style-type: none"> – Shimadzu: part no. 228-37546-92 (Deep-well MTP Rack, DWP-96) – UltiMate 3000: Thermo part no. 6820.4083 (30 – 36 mm height) 	<ul style="list-style-type: none"> – Waters ACQUITY UPLC® system with sample manager, solvent manager, and column oven: <ul style="list-style-type: none"> – Classic (loop-based) – I-Class (FTN) – H-Class (FTN) – Injection volume: <ul style="list-style-type: none"> – loop: 20 µL – FTN: 10 µL 	

LC-MS instrument status

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

Analytical columns

UHPLC* – Option 1	
Analytical column	AbsoluteIDQ® p180 kit UHPLC column: biocrates product number 9120052121193 (Precolumn not required)

UHPLC* – Option 2	
Analytical column	Waters ACQUITY UPLC BEH C18 1.7µm, 2.1 x 75mm (186005604)
Precolumn	Waters ACQUITY BEH C18 1.7µm VANGUARD (186003975)
Precolumn holder	Already included in the Waters VANGUARD precolumn package

* UHPLC not compatible with SCIEX 4000 series

HPLC	
Analytical column	Agilent Zorbax Eclipse XDB C18 3.5µm, 3.0 x 100mm (961967-302)
Precolumn	Phenomenex SecurityGuard, C18, 4 x 3mm (AJO-4287)
Precolumn holder	Phenomenex SecurityGuard Cartridge (KJO-4282)

Solvents and chemicals

Solvents and chemicals	Purity
Ethanol, methanol, water, acetonitrile, isopropanol	LC-MS grade
Formic acid	LC-MS grade (e.g. Honeywell Fluka™ 56302-50ML) Fresh! New bottle or opened within past 6 months
Phenyl isothiocyanate (PITC)	99%, for protein sequencing (e.g. Sigma-Aldrich 317861) Fresh! New bottle or opened within past 6 months
Pyridine	Purity >99.5% Fresh! New bottle or opened within past 6 months
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* > Requires nitrogen supply > Must be in a fume hood	Examples: <ul style="list-style-type: none"> – Porvair blowdown evaporator MiniVap® – Techne (FSC496D) – Sample concentrator from BenchTop Lab Systems (96 samples, BT1604) – VLM evaporators – Organomation MICROVAP microplate evaporator 	Examples: <ul style="list-style-type: none"> – 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)

	– Biotage TurboVap® 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, 2.5, and 10 mL tips) or similar electronic model – Single channel: volume range 10 µL – 1000 µL – 8-Channel: 10 µL – 150 µL	
Vortexer	Any model	
Balance	Accuracy < 1 mg	
Solvent bottles	50 – 1000 mL	

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

Software

Agilent	SCIEX	Thermo Scientific	Waters
MS software			
MassHunter version B.09 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"> – Oracle® database: <ul style="list-style-type: none"> – Option 1 (recommended): full commercial version, updates and support provided by Oracle. Expert required. – Option 2: Oracle Database Express Edition (Oracle XE), free of charge, provided by biocrates. No updates and support provided by Oracle. – MetIDQ™ software (provided by biocrates) – Windows 7 or later, 64-bit architecture – Microsoft .NET Framework 4.5.2 or later – Microsoft Visual C++ redistributables (32 & 64 bit each): 2005, 2008, 2010, 2012, and 2013 			