

Cell Lysis: Reagents	Procedure
Lysis buffer 50 mM MOPS (4-Morpholinepropanesulfonic acid) 6 M urea 2 M thiourea	resuspend pellet in 200 µL Lysis buffer transfer into 1.5 mL Eppendorf tube
Reduction and Alkylation: Reagents	Reduction and Alkylation: Procedure
0.2 M DTT in 50 mM MOPS (stored @ -20°C) 1,4-dithiothreitol MW 154.25 g/mol = 15 mg in 500 µL	Add 8 µL of 0.2 M DTT <input type="text"/>
0.4 M iodoacetamide in 0.1 M MOPS MW 184.96 g/mol = 37 mg in 500 µL	Incubate for 15 min @ 37 °C on a shaker with 500 rpm Cool down the sample to room temperature Add 8 µL of 0.4 M iodoacetamide <input type="text"/>
Trypsin digest: Reagents	Trypsin digest: Procedure
Trypsin, sequencing grade modified V511C, Promega; stored @ -80 °C aliquots of 20 µg (40 µL, 0.5 µg/µL)	Check pH with pH paper (might add 3 µL 1 M NaOH) add 800 µL 50 mM MOPS <input type="text"/>
Pre-HILIC	Pre-HILIC
Eppendorf table top centrifuge 5417R	Incubate @ 37 °C on a shaker with 500 rpm overnight
HILIC	Pre-HILIC
Harvard Apparatus GmbH Macro SpinColumns, Hydrophilic Packing Material Qty. of 96; 744305	Reduce volume in speed vac (45 °C) to about 200 µL (about 1 h) add 800 µL '100 % Ethanol' + 50 mM ammonium formate to sample
Conditioning Buffer pH 6 50 mM ammonium formate 40 % acetonitrile	HILIC-column preparation
Loading Buffer pH 6 50 mM ammonium formate 80 % acetonitrile	add 300 µL 100 % methanol to column centrifuge for 2 min @ 1100 rcf discard flowthrough add 300 µL dH ₂ O
	centrifuge for 2 min @ 800 rcf (~3000 rpm) <input type="text" value="3x"/>
	discard flowthrough add 600 µL Conditioning Buffer pH 6 transfer 325 µL of slurry into second tube let equilibrate for approximately 1 hour
	centrifuge for 2 min @ 800 rcf (~3000 rpm) add 300 µL Loading Buffer pH 6
	centrifuge for 2 min @ 800 rcf (~3000 rpm) <input type="text" value="3x"/>
	discard flowthrough
HILIC	HILIC
Elute HILIC 2 % formic acid, HPLC water	Centrifuge sample for 3 min at 12'000 rpm (4 °C) transfer supernatante into new 1.5 mL eppendorf tube add 400 µL of sample to HILIC column store second half (600 µL) of sample at -20 °C
MS-loading buffer A 2 % acetonitrile 0.2 % formic acid	centrifuge for 2 min @ 800 rcf (~3000 rpm) discard flowthrough add 300 µL Loading Buffer pH 6
	centrifuge for 2 min @ 800 rcf (~3000 rpm) <input type="text" value="2x"/>
	discard flowthrough, add HILIC columns to a new 2 mL tube add 400 µL Elute HILIC centrifuge for 2 min @ 800 rcf (~3000 rpm) dry under vaccum 45 °C
MassSpec	MassSpec
	resuspend in 20 µL MS-loading buffer A nanodrop A(220 nm)