

# Single-cell Proteomics Preparation for Mass Spectrometry Analysis using Freeze-Heat Lysis and an Isobaric Carrier

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## Materials

Name	Company	Catalog Number	Comments
384-well plate, standard PCR plate	Thermo Fisher Scientific	AB1384	Polypropylene plates should be used, if need to substitute. If substituted, levels of potential polymer contamination from other plates should be assessed prior to SCoPE2 preparation.
Acetonitrile (for preparation of buffers), Optima LC-MS/MS grade	Fisher Scientific	A955-1	This acetonitrile is used for any buffers, namely the 50% acetonitrile that is used to pass through wells after passing through the carrier and reference when combining SCoPE2 sets. Caution: Acetonitrile is a flammable liquid. It can irritate skin, eyes, respiratory tract, and central nervous system. Be sure to wear personal protective equipment. Handle under a chemical fume hood.
Acetonitrile (for preparation of TMT ), anhydrous, 99.8%	Sigma Aldrich	271004-100ML	This acetonitrile is used to resuspend TMT labels at the manufacturer concentration. Caution: Acetonitrile is a flammable liquid. It can irritate skin, eyes, respiratory tract, and central nervous system. Be sure to wear personal protective equipment. Handle under a chemical fume hood.
Adhesive PCR plate foils	Thermo Fisher Scientific	AB0626	
Autosampler vial screw thread caps	Thermo Fisher Scientific	C5000-51B	
Autosampler vial spinner (i.e. myFuge 5)	MTC Bio	C2595	This model does not have any speed control. It is simply used to collect liquid at the bottom of autosampler vials.
Benzonase nuclease	Sigma Aldrich	E1014-25KU	

Clear glass screw thread vials, 9 mm	Thermo Fisher Scientific	60180-509	
Formic Acid, Pierce, LC-MS/MS grade	Thermo Fisher Scientific	85178	Caution: Formic acid is a flammable liquid. It can cause serious eye damage or skin burns. Be sure to wear personal protective equipment. Handle in a well-ventilated area.
Glass autosampler inserts, 9 mm	Thermo Fisher Scientific	C4010-630	
Hydroxylamine (HA), 50% wt/vol	Sigma Aldrich	467804-50ML	Caution: Hydroxylamine can cause skin irritation. Be sure to wear personal protective equipment.
Mantis microfluidic chips, low-volume 3PFE chips	Formulatrix	MCLVPR2	These chips are meant to be used with the Mantis microfluidics liquid handler to dispense organic solutions. These can be used to dispense TMT labels.
Mantis microfluidic chips, low-volume silicone chips	Formulatrix	MCLVS12	These chips are meant to be used with the Mantis microfluidics liquid handler to dispense aqueous solutions. These cannot be used to dispense TMT labels.
Mantis microfluidic liquid handler	Formulatrix		Liquid handler can be substituted. However, it is important to check if the system is compatible with 100% acetonitrile (as in the case of TMT labels), and that the system does not introduce polymer contamination or other type of contamination into the samples.
Mantis PCR plate adapter with wide conical pins for automated plate handling	Formulatrix	232400	
MassPREP peptide mixture	Waters	186002337	Mixture of nine nontryptic peptides
PCR tube spinner (i.e., 16-place microcentrifuge for 0.2 mL tubes)	USA Scientific	2621-0016	This model does not have any speed control. It is simply used to collect liquid at the bottom of tubes.
PCR tubes: TempAssure 0.2 mL PCR 8-tube strips	USA Scientific	1402-3900	If substituted, levels of potential polymer contamination from other plates should be assessed prior to SCoPE2 preparation.
Phosphate-buffered saline (PBS), 10x, pH 7.4, RNase-free	Thermo Fisher Scientific	AM9625	
Plate spinner (i.e., PlateFuge microcentrifuge)	Benchmark Scientific	Model C2000	This model does not have any speed control. It is simply used to collect liquid at the bottom of wells.
Thermal Cycler (i.e., C1000 Touch with 384-well module)	Biorad	1851138	
TMTpro 16plex Label Reagent Set, 1 x 5 mg	Thermo Fisher Scientific	A44520	
Triethylammonium bicarbonate (TEAB), 1 M, pH 8.5	Sigma Aldrich	T7408100ML	
Trypsin Gold Mass Spectrometry Grade	Promega	V5280	This is trypsin is of very high purity. Other trypsin reagents can vary in their levels of purity. Level of purity is important for achieving positive SCoPE2 results. Caution: Trypsin can cause skin, respiratory, and eye irritation. Be sure to wear personal protective equipment. Handle under a chemical fume hood.
Vortex (Analog vortex mixer)	VWR	Model 58816-121	

Water bath sonicator (i.e., 2.8 L ultrasonic cleaner with digital timer)	VWR	97043964	
Water, Optima LC-MS/MS grade	Fisher Scientific	W6-1	All solutions that need to be diluted or made are recommended to be made with mass-spectrometry grade water. Any dilutions and/or master mixes should be made fresh. Contamination resulting from lower-grade water can negatively affect peptide detection and single-cell data quality .