PREOMICS

BeatBox Tissue Kit 96x & iST 8x

Formalin-fixed, paraffin-embedded (FFPE) tissue

Introduction

Formalin-fixed, paraffin-embedded (FFPE) tissues are a valuable source of information, but also a challenging matrix for bottom-up proteomic studies. The PreOmics® FFPE sample preparation solution provides an easy-to-use and robust workflow that allows a deep insight into the tissue proteome in a few steps with minimal hands-on time. For sample-specific protocols and optimization, visit www.preomics.com/resources or contact info@preomics.com.

Protocol

This protocol outlines the complete workflow for proteomic sample preparation, including BeatBox®-based tissue homogenization and protein extraction, followed by protein reduction, alkylation, digestion, and subsequent peptide purification. It specifically describes the preparation of FFPE samples using the BeatBox Tissue Kit 96x (P.O.00121), iST 8x kit (P.O.00001), and additional washing buffer WASH 0 (P.O.00095). For all required labware, refer to the "Pre-Requisites" section below.

Material

Component	Cap	Quantity	Buffer Properties				Description	Storage
iST 8x (P.O. 00001)			Organic	Acidic	Basic	Neutral		
DIGEST	red	2x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND	yellow	1x 2 mL				●	For reconstituting lyophilized proteolytic enzymes.	RT
LYSE	brown	1x 1 mL			●		For denaturing, reducing, and alkylating proteins.	RT
STOP	black	1x 1 mL	●	●			For stopping enzymatic activity.	RT
WASH 1	blue	1x 2 mL	●	●			For removing hydrophobic contaminants.	RT
WASH 2	green	1x 2 mL		●			For removing hydrophilic contaminants.	RT
ELUTE	pink	1x 2 mL	●		●		For eluting peptides from the cartridge.	RT
LC-LOAD	white	1x 1 mL		●			For loading peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		8x					Cartridges with SPE sorbent for cleaning up peptides from 1–100 µg protein starting material.	RT
WASTE TUBE		8x					2 mL tube for collecting waste after washing steps.	RT
COLLECTION TUBE		8x					1.5 mL tube for collecting peptides after elution.	RT
ADAPTER		8x					Enables a cartridge to be placed into a tube.	RT
Additional reagents, kits and instruments from PreOmics:								
WASH 0 (P.O.00095)	light pink	10 mL	●	●			For removal of remaining paraffin. Please order 1x WASH 0 buffer 10 mL (P.O.00095) from PreOmics, or ask for the buffer recipe at info@preomics.com.	RT
BeatBox instrument (P.O.00144)							Tissue homogenizer with accessory kit.	
BeatBox Tissue Kit 96x (P.O.00121)							Consumables for protein extraction on the BeatBox in 96 well format.	

Pre-Requisites

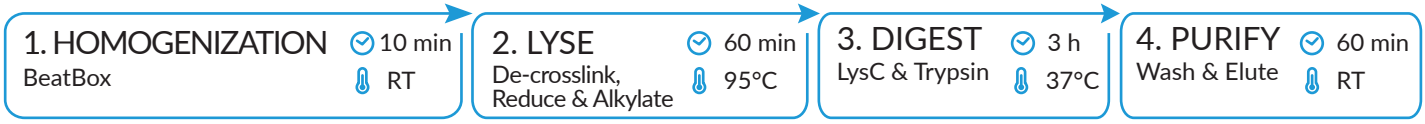
Common lab equipment is required for the sample preparation.

Consumables	Quantity and Description
CAP STRIPS	Ensures tight sealing of the BEATBOX 96x PLATE during sample homogenization and protein extraction. Cap Strips, flat 10x12 PCR clean; Eppendorf, catalogue number EU&US: 0030124847.
REACTION TUBE	1.5 mL microcentrifuge low protein binding tubes are recommended (e.g., Eppendorf Protein LoBind® Tubes, catalogue number EU: 0030108116, US: 022431081).

Equipment	Quantity and Description
PIPETTE	Standard single-channel pipettes can be used.
PLASTIC TWEEZERS	For tissue transfer into BEATBOX 96x PLATE.
HEATING SHAKER	Two separate devices are recommended to support the different temperatures of the LYSE and DIGEST steps.
CENTRIFUGE	Swing-bucket centrifuge for multi-well plate is required for protein extraction, and benchtop centrifuge for 1.5 or 2 mL tubes is required for the iST workflow.
VACUUM EVAPORATOR	To evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.

Sample	Quantity and Description
FFPE TISSUE	Either deparaffinized tissue or full formalin-fixed, paraffin-embedded (FFPE) curls without deparaffinization. FFPE tissue curl, 10 µm thickness. The FFPE curl thickness can be adjusted to suit your needs; see *NOTE1* for further information. For other FFPE sample types, contact PreOmics for adapted protocols.

Procedure



Method

1. HOMOGENIZATION *NOTE2*

For a detailed description and graphical representation on how to use the BeatBox, please refer to the BeatBox Quick Start Manual 96x.

1.1. Remove the **SILICONE MAT** from the **BEATBOX 96w PLATE** while keeping the **METAL SHEET** attached to the base of the **BEATBOX 96w PLATE**. If the BeatBox plate is only partially filled (e.g., 48 wells), the silicone mat can be cut to the appropriate number of wells by using scissors.

1.2. Prefill wells with 50 µL **LYSE**  . *NOTE1*

1.3. Add FFPE TISSUE into the well of the **BEATBOX 96w PLATE** using PLASTIC TWEEZERS (using metallic tweezers can cause loss of Gyuto Beads). To avoid cross contaminations during sample transfer, cover all remaining wells with **SILICONE MAT** or CAP STRIPS.

1.4. Close sample-containing wells with CAP STRIPS and remove the **METAL SHEET** from the base of the **BEATBOX 96w PLATE**. *CRITICAL* Make sure the wells are tightly sealed.

1.5. Place the **BEATBOX 96w PLATE** on the **PLATE ADAPTER** of the BeatBox accessory kit, insert the **PLATE** and **ADAPTER** assembly into the **GARAGE,** and start the BeatBox run with HIGH power settings for 10 min.

1.6. After the BeatBox run is completed, remove the **GARAGE** from the instrument, and **BEATBOX 96w PLATE** from the **PLATE ADAPTER**.

1.7. Spin down the **BEATBOX 96w PLATE** (RT; max. 300 rcf; 30 sec).

2. LYSE

2.1. Place the **BEATBOX 96w PLATE** on a pre-heated HEATING SHAKER (80-95 °C; 1,000 rpm; 1 h). *NOTE3*

2.2. Place the **BEATBOX 96w PLATE** on the **GYUTO BEAD COLLECTION RACK** and let samples cool down to room temperature. If intact tissue is still visible, repeat BeatBox run (steps 1.5–1.7) and optional, the boiling step (steps 2.1–2.2). Make sure that wells are tightly sealed.

2.3. Remove the **BEATBOX 96w PLATE** from the **GYUTO BEAD COLLECTION RACK** and spin down the **BEATBOX 96w PLATE** (RT; max. 300 rcf; 30 sec).

2.4. Place the **BEATBOX 96w PLATE** back on the **GYUTO BEAD COLLECTION RACK** and transfer the homogenate into a clean REACTION TUBE for subsequent processing or analysis workflows.
CRITICAL The hardened paraffin might form a ring in the wells of the **BEATBOX 96w PLATE** and should be left in the plate when transferring the homogenate.

3. DIGEST

3.1. Optional: Measure protein concentration. BCA-RAC assay can be used. Visit our FAQ for recommendations on protein quantitation assays. *CRITICAL* The samples should be vortexed prior to protein quantitation. Do not centrifuge or allow particles to settle.

3.2. Add the homogenate with up to 100 µg of extracted protein in a final volume of 50 µL into a REACTION TUBE. If the volume is <50 µL, fill up to 50 µL with **LYSE**  .

3.3. Optional: Spin down lyophilized enzyme mix in DIGEST tube (RT; max. 300 rcf; 10 sec).

3.4. Add 210 µL **RESUSPEND**  to **DIGEST**  (1 tube for 4 reactions), shake (RT; 500 rpm; 10 min) and pipette up/down. *NOTE4*

3.5. Add 50 µL **DIGEST**  to each REACTION TUBE and place it in a pre-heated HEATING SHAKER (37°C; 500 rpm; 3 h).

3.6. Spin down droplets (RT; 300 rcf; 30 sec).

3.7. Add 100 µL **STOP**  to REACTION TUBE, shake (RT; 1000 rpm; 1 min), and pipette up/down.*SP*

3.8. Assemble **ADAPTER** and **CARTRIDGE** onto the top of **WASTE TUBE**. Label all tubes and transfer sample to the **CARTRIDGE**.

4. PURIFY

4.1. Spin **CARTRIDGE** in a centrifuge at 3,800 rcf for 1–3 min. If needed, adjust time to ensure complete flow-through.

4.2. Add 200 µL **WASH 0** to **CARTRIDGES,** repeat step 4.1. (WASH 0 steps are optional for deparaffinized tissue. Continue with step 4.4 otherwise).

4.3. Add again 200 µL **WASH 0** to **CARTRIDGES,** repeat step 4.1.

4.4. Add 200 µL **WASH 1** to **CARTRIDGES,** repeat step 4.1.

4.5. Add 200 µL **WASH 2** to **CARTRIDGES,** repeat step 4.1.

4.6. Transfer assembled **ADAPTER** and **CARTRIDGE** onto clean **COLLECTION TUBE**. Discard **WASTE TUBE**. Label all tubes.

4.7. Add 100 µL **ELUTE** to **CARTRIDGES**, repeat step 4.1, keep flow-through in **COLLECTION TUBE**.

4.8. Repeat step 4.7, keep flow-through in the same **COLLECTION TUBE**.

4.9. Discard **CARTRIDGES** and place **COLLECTION TUBE** in a VACUUM EVAPORATOR (45°C; until completely dry). *SP*

4.10. Reconstitute peptides by adding **LC-LOAD** to **COLLECTION TUBE**. Adjust the volume according to specific requirements. For example, add 50 µL **LC-LOAD** to 100 µg protein starting material.

4.11. Sonicate **COLLECTION TUBE** in an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min).

4.12. Spin **COLLECTION TUBE** in a CENTRIFUGE (RT; 16,000 rcf; 5 min), transfer the supernatant to a clean vial and avoid touching the bottom of the collection tube during transfer. *NOTE5*

NOTE1	FFPE curls with a thickness of 10–20 µm are compatible with the protocol. When working with 20 µm curls or excess paraffin, we recommend 100 µL LYSE buffer. For sample homogenization, your own buffer (see FAQs for composition compatibility and limitations) can be used. If your lysis buffer contains >0.1% SDS, SDS removal with the SP3-iST add-on is required before continuing with the iST protocol. For a modified protocol using the SP3-iST kit, contact info@preomics.com.
NOTE2	SINGLE USE ONLY: Each well and GYUTO BEAD should be used only once. Unused wells of the BEATBOX 96w PLATE may be used at a later timepoint. Total runtime of the BEATBOX 96w PLATE is recommended not to exceed 40 minutes, regardless of the settings used.
NOTE3	The sample temperature reached inside the wells may vary between different heating shaker models. At very high temperatures, the CAP STRIP may burst open due to high vapor pressure. To avoid sample loss, perform a test run with lysis buffer to identify the highest possible temperature for your heating shaker setup. Please do not use a heated lid.
NOTE4	Resuspended DIGEST can be stored for up to two weeks at 4°C. For longer storage periods, visit our FAQ.
NOTE5	At this point, peptide concentration can be measured or the sample directly injected for LC-MS analysis. Visit our FAQ for recommendations on peptide quantitation assays.
SP - Storage Point:	Peptides can be frozen at -20°C for two weeks. Dried peptides before reconstitution in LC-LOAD can also be stored long-term at -80°C.

Data analysis

Consider the following as fixed modifications in your database search:

MODIFICATION	DESCRIPTION	COMPOSITION	SPECIFICITY	MASS	UNIMOD #
ALKYLATION	Carbamidomethyl on cysteine	C_2H_3NO	[C]	+57Da	4

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