PREOMICS

BeatBox Tissue Kit 96x & iST 96x

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 and 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 describes high-throughput FFPE sample preparation in 96-well format using the BeatBox Tissue Kit 96x (P.O.00121), iST 96x HT kit (P.O.00150), and washing buffer WASH 0 (P.O.00095). The iST 96x HT kit can be replaced by the iST 96x kit (P.O.00027) or the iST 96x HT DV kit (P.O.00198). For additional labware needed, please see the "Pre-Requisites" section below.

Material

Component	Cap	Quantity	Buffer Properties				Description	Storage
iST 96x HT (P.O. 00150)			Organic	Acidic	Basic	Neutral		
DIGEST	🔴	24x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND	🟡	1x 20 mL				●	For reconstituting lyophilized proteolytic enzymes.	RT
LYSE	🟤	1x 20 mL			●		For denaturing, reducing, and alkylating proteins.	RT
STOP	⚫	1x 15 mL	●	●			For stopping enzymatic activity.	RT
WASH 1	🔵	1x 25 mL	●	●			For removing hydrophobic contaminants.	RT
WASH 2	🟢	1x 25 mL		●			For removing hydrophilic contaminants.	RT
ELUTE	🟣	1x 25 mL	●		●		For eluting peptides from the cartridge.	RT
LC-LOAD	⚪	1x 20 mL		●			For loading peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		96x					Cartridges with SPE sorbent for cleaning up peptides from 1–100 µg protein starting material. Racked in adapter plate and closed with silicone mat.	RT
WASTE PLATE		1x					Deep well plate for collecting waste after washes.	RT
COLLECTION PLATE		1x					LoBind® plate for collecting peptides after elution. The plate has a max. working volume of 150 µL, and can be used after validating the workflow for elution with lower buffer volumes. Alternatively, use ELUTE PLATE (see Pre-Requisites section below).	RT
ADAPTER PLATE		1x					Enables a cartridge to be placed on top of 96w plates.	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)	◯	4x 10 mL	●	●	For removal of remaining paraffin Please order 4x 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

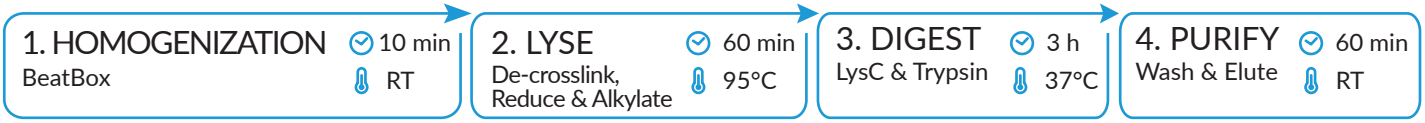
Common lab equipment is required for the sample preparation.

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 PLATE	For protein digestion, samples may be handled in any reaction vessel >450 µL, but a 96x deep well plate is recommended (e.g., Eppendorf Deepwell plate 96/500 µL Protein LoBind®, catalogue number EU: 0030504100, US: 951032107).
ELUTE PLATE	96 deep well plate with >250 µL for elution of peptides from cartridge (e.g., Eppendorf Deepwell plate 96/500 µL Protein LoBind®, catalogue number EU: 0030504100, US: 951032107).
SEALING MAT	Prevents sample contamination and evaporation during digestion (e.g. Eppendorf Sealing Mat, catalogue number EU&US: 0030127978).
96 WELL PLATES	96 deep well & 96 well skirted plates to balance WASTE & COLLECTION PLATE in centrifuge.

Equipment	Quantity and Description
PIPETTE	Standard single-channel pipettes can be used. It is recommended to replace them e.g. by dispenser or multichannel pipettes where possible.
PLASTIC TWEEZERS	For tissue transfer into BeatBox 96x PLATE.
HEATING SHAKER	Two heating shakers for multi-well plates are recommended to support protein denaturation and digestion.
CENTRIFUGE	Swing-bucket centrifuge for 96 well plate is required for spin-down of homogenate and peptide loading, washing, and elution.
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

Critical Note: For automation processes, only plates with low protein binding properties should be used as buffer reservoirs to avoid polymer contamination. Contact us at info@preomics.com for advice on buffer and plasticware usage with liquid handling platforms.

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 and 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 the **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 (step 1.5-1.7) and optional, the boiling step (step 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 REACTION PLATE for subsequent processing or analysis workflows. *CRITICAL* The hardened paraffin might form a ring in thewells 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 PLATE. 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 well, close REACTION PLATE with a SEALING MAT and place plate on 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 each well, shake (RT; 1000 rpm; 1 min), and pipette up/down. *SP*

3.8. Place CARTRIDGES in ADAPTER PLATE on WASTE PLATE. Label all wells and transfer sample to the **CARTRIDGE**. *NOTE5*

4. PURIFY

4.1. Spin **CARTRIDGES** in a centrifuge at 2,250 rcf for 1–3 min. If needed, adjust time to ensure complete flow-through.

4.2. Discard flow through collected in **WASTE PLATE** before continuing with next step.

4.3. Add 200 µL **WASH 0** ○ to **CARTRIDGES,** repeat step 4.1 & 4.2 (WASH 0 steps are optional for deparaffinized tissue. Continue with step 4.6 otherwise).

4.4. Add again 200 µL **WASH 0** ○ to **CARTRIDGES,** repeat step 4.1 & 4.2

4.5. Add 200 µL **WASH 1** ● to **CARTRIDGES,** repeat step 4.1 & 4.2.

4.6. Add 200 µL **WASH 2** ● to **CARTRIDGES,** repeat step 4.1 & 4.2

4.7. Discard **WASTE PLATE**. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of the ELUTE PLATE. Label plate and wells. Alternatively, use the supplied **COLLECTION PLATE** after validating the workflow for elution with lower buffer volumes. See “Kit Contents” for details.

4.8. Add 100 µL **ELUTE** ● to **CARTRIDGES,** repeat step 4.1, keep flow-through in ELUTE PLATE.

4.9. Repeat step 4.8, keep flow-through in the same ELUTE PLATE.

4.10. Discard **CARTRIDGES** and place ELUTE PLATE in a VACUUM EVAPORATOR (45 °C; until completely dry). *SP*

4.11. Reconstitute peptides by adding **LC-LOAD** ○ to ELUTE PLATE. Adjust the volume according to specific requirements. For example, add 50 µL **LC-LOAD** to 100 µg protein starting material.

4.12. Sonicate ELUTE PLATE in an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min).

4.13. Spin plate in a CENTRIFUGE as follows:

- User-provided ELUTE PLATE: RT; maximum rcf recommended by manufacturer; 5–15 min.
- **COLLECTION PLATE**: RT; 2,250 rcf; 15 min.

Transfer the supernatant to a clean plate and avoid touching the bottom of the well during transfer. *NOTE6*

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 Alternatively, use **ADAPTERS** to place single **CARTRIDGES** on top of 2 mL tubes. Consult the corresponding protocol BeatBox and iST for FFPE Tissues 8x for subsequent steps.

NOTE6 At this point, peptide concentration can be measured or the sample can be directly injected for LC-MS analysis. Visit our FAQ for recommendations on peptide quantitation assays. The silicone mat provided with the kit is incompatible with autosamplers.

SP - Storage Point At this point, close the peptide containing plate or **CARTRIDGE** using the silicone mat. Peptides can be frozen at -20°C for two weeks. Dried peptides, prior to 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

For answers to frequently asked questions, please visit our FAQ page at www.preomics.com/faq.

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