PREOMICS

BeatBox Tissue Kit 96x coupled to 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 96x 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, questions, or optimization, contact info@preomics.com or visit PreOmics FAQs.

Protocol

The protocol includes all steps to perform a complete proteomic sample preparation including BeatBox-based sample homogenization and protein extraction, reduction and alkylation, and digestion of proteins followed by peptide purification. The following protocol describes high-throughput FFPE sample preparation in 96-well format using the BeatBox Tissue Kit 96x and the iST 96x kit (iST 96x P.O.00027; iST-HT 96x P.O.00150). For additional labware needed, please see the "Pre-Requisites" section below.

Material

Component	Cap	Quantity	Buffer Properties				Description	Storage
iST Kit 96x (P.O. 00150 iST-HT 96x)			Organic	Acidic	Basic	Volatile		
DIGEST	red	24x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND	yellow	1x 20 mL					Reconstitutes lyophilized proteolytic enzymes.	RT
LYSE	brown	1x 20 mL			●		Denatures, reduces and alkylates proteins.	RT
STOP	black	1x 15 mL	●	●		●	Stops the enzymatic activity.	RT
WASH 1	blue	1x 25 mL	●	●		●	Cleans peptides from hydrophobic contaminants.	RT
WASH 2	green	1x 25 mL		●		●	Cleans peptides from hydrophilic contaminants.	RT
ELUTE	pink	1x 25 mL	●		●	●	Elutes the peptides from the cartridge.	RT
LC-LOAD	white	1x 20 mL		●		●	LC-MS compatible peptide reconstitution buffer.	RT
CARTRIDGE		96x					Cartridge for 1 to 100 µg protein starting material.	RT
WASTE PLATE		1x					Deep well plate for collecting waste after washes.	RT
MTP PLATE		1x					LoBind plate for collecting peptides after elution.	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	light pink	40 mL	●	●		●	Cleans peptides from remaining paraffin. Please order the buffer in addition to the iST and BeatBox kits from PreOmics (4x WASH 0 Buffer 10 mL (P.O.00095) with the comment "FFPE protocol") or ask for the buffer recipe at info@preomics.com.	RT
BeatBox instrument							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

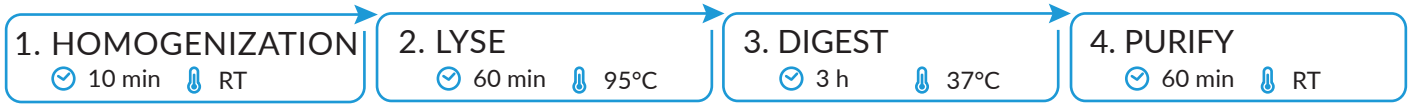
Common lab equipment is required for the sample preparation. Always make sure to be equipped with personal protective equipment (e.g. safety glasses, lab coat and gloves). When working with large quantities of solvents, work should be carried out under a fume hood. Always consult the corresponding safety data sheet (SDS) upfront.

Consumables	Quantity and Description
CAP STRIPS for BEATBOX 96x PLATE	Ensures tight sealing of the BEATBOX 96x PLATE during sample homogenization and protein extraction. Cap Strips, flat 10x12 PCR clean; Eppendorf Cat. -Nr.: 0030124847.
DEEP WELL 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®, Cat.-Nr. 0030504100).
SEALING MAT	Prevents sample contamination and evaporation during digestion (e.g. Eppendorf Sealing Mat, Cat.-Nr. 0030127978).
96 WELL PLATES	96 deep well & 96 well skirted plates to balance WASTE & MTP 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 96 well plate.
THERMOSHAKER	Two separate devices are recommended to support the different temperatures of the LYSE and DIGEST steps.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.
VACUUM CONCENTRATOR	To evaporate volatile buffers from the eluate before LC-MS.
CENTRIFUGE	Swing-bucket centrifuge for 96 well plate and adequate counterweight are required for spin-down of homogenate and peptide loading, washing, and elution.

Equipment	Quantity and Description
SAMPLE	10 µm curl from FFPE tissue. Either deparaffinized tissue or full FFPE curl without deparaffinization.

Procedure



Method

1. BEATBOX HOMOGENIZATION *NOTE1*

Start Protocol either with deparaffinized tissue or full FFPE curl without deparaffinization.

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** . *NOTE2*

1.3. Add SAMPLE 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).

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

1.9. Place the **BEATBOX 96w PLATE** on the GYUTO BEAD COLLECTION RACK and let samples cool down to RT.
If intact tissue is still visible, repeat BeatBox run (step 1.5.-1.7.) and optional, the boiling step (step 1.8. – 1.9.). Make sure that wells are tightly sealed.

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

1.11. Place the **BEATBOX 96w PLATE** back on the GYUTO BEAD COLLECTION RACK and transfer the homogenate into a DEEP WELL 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.

2. DIGEST

2.1. Add the homogenate with up to 100 µg of extracted protein in a final volume of 50 µL into a DEEP WELL PLATE. If the volume is < 50 µL, fill up to 50 µL with **LYSE** .

2.2. Add 210 µL **RESUSPEND** to **DIGEST** (1 vial for 4 reactions) and shake (RT; 500 rpm; 10 min).

2.3. Add 50 µL **DIGEST** to each well, place a SEALING MAT (Eppendorf Sealing Mat) on the DEEP WELL PLATE and transfer it to a pre-heated THERMOSHAKER (37°C; 500 rpm; 3 hours).

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

2.5. Add 100 µL **STOP** to each sample, shake (RT; 1000 rpm; 1 min/pipette up/down). *SP*

3. PURIFY

3.1. Use **ADAPTER PLATE** to place **CARTRIDGES** on top of **WASTE PLATE** or use **ADAPTERS** to place single **CARTRIDGES** on top of tubes and transfer samples to the **CARTRIDGES**. Be careful not to damage the bottom layer of the **CARTRIDGES**. *NOTE4* *AM*

3.2. Spin **CARTRIDGES** in a centrifuge at 2,250 rcf for 1-3 min (if needed, adjust time to ensure complete flow-through of the wash-liquid).

3.3. Add 200 µL **WASH 0** to **CARTRIDGES** (WASH 0 steps are optional for deparaffinized tissue. Continue with step 3.8. otherwise).

3.4. Spin **CARTRIDGE** in a centrifuge at 2,250 rcf for 1-3 min (if needed, adjust time to ensure complete flow-through).

3.5. Discard flow through in case of using **WASTE PLATE** before continuing with next step.

3.6. Add again 200 µL **WASH 0** to **CARTRIDGES**.

3.7. Repeat step 3.4. and 3.5.

3.8. Add 200 µL **WASH 1** to **CARTRIDGES.**

3.9. Repeat step 3.4. and 3.5.

3.10. Add 200 µL **WASH 2** to **CARTRIDGES.**

3.11. Repeat step 3.4.

3.12. Discard **WASTE PLATE**. Use **ADAPTER PLATE** to place **CARTRIDGE** on top of **MTP PLATE**. Label all wells.

3.13. Add 100 µL **ELUTE** to **CARTRIDGES.**

3.14. Spin **CARTRIDGES** in a centrifuge at 2,250 rcf for 1-3 min and collect it in MTP PLATE.

3.15. Repeat step 3.13. - 3.14., keep flow-through in the same **MTP PLATE**.

3.16. Discard **CARTRIDGES** and place **MTP PLATE** in a vacuum concentrator (45 °C; until completely dry). *SP*

3.17. Add 50 µL **LC-LOAD** to **MTP PLATE** and shake (RT; 500 rpm; 5 min).

3.18. Optional: Spin **MTP PLATE** in a CENTRIFUGE to remove particles (maximum rcf recommended by manufacturer, 5 min).

3.19. Check and adjust the peptide concentration to suit your LC-MS setup.

NOTE1 SINGLE USE ONLY: Kits components cannot be re-used

NOTE2 When working with larger sample input (e.g. 20 µm curls) or excess of paraffin, we recommend 100 µL LYSE buffer. For sample homogenization, your own buffer (please 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, please contact info@preomics.com.

NOTE3 The sample temperature reached inside the wells may vary between different thermoshaker models. At very high temperatures the cap strip may burst open due to high vapor pressure. To avoid loss of sample, perform a test run with lysis buffer to identify the highest possible temperature for your thermoshaker setup. Please do not use a heated thermoshaker lid.

NOTE4 When working with single CARTRIDGES consult the corresponding protocol for iST Kit 8x.

SP - Storage Point At this point, close the peptide containing tube. Peptides can be frozen at -20 °C (max. two weeks) or at -80 °C for long-term storage.

AM - Automation PreOmics also offers iST kits compatible with positive pressure devices. Please contact info@preomics.com for more information and a modified protocol.

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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