PREOMICS

ENRICH-iST Kit 8x

Mammalian blood plasma and serum

Introduction

Blood plasma and serum can be challenging matrices for proteomic analyses due to their complexity and high dynamic range. The PreOmics ENRICH-iST technology provides a novel enrichment workflow that allows a deep insight into the blood proteome of mammalian species. Combined with the proven iST-BCT kit, it offers a fast, robust and automatable sample preparation solution. For specific protocols and optimization, visit https://www.preomics.com/resources or contact info@preomics.com.

Kit Contents

The kit contains all core components to perform a complete proteomic sample preparation - protein enrichment, alkylation, reduction, digestion and peptide clean-up. For additional labware needed, please see the "Pre-Requisites" section below.

Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Volatile		
Part 1: ENRICH								
EN-BEADS	○	1x 0.25 mL					Paramagnetic beads that bind plasma proteins.	2-8 °C
EN-WASH	○	1x 6 mL					For removing the bead storage solution.	2-8 °C
EN-BIND	○	1x 4 mL					Facilitates protein binding onto the beads.	2-8 °C
Part 2: iST-BCT								
DIGEST	○	2x					Trypsin/LysC mix to digest proteins.	-20 °C
RESUSPEND-BCT	○	1x 2 mL					Reconstitutes lyophilized proteolytic enzymes.	RT
LYSE-BCT	○	1x 1 mL					Denatures, reduces and alkylates proteins.	RT
STOP	○	1x 1 mL	●	●		●	Stops the enzymatic activity.	RT
WASH 1	○	1x 2 mL	●	●		●	Cleans peptides from hydrophobic contaminants.	RT
WASH 2	○	1x 2 mL		●		●	Cleans peptides from hydrophilic contaminants.	RT
ELUTE	○	1x 2 mL	●		●	●	Elutes the peptides from the cartridge.	RT
LC-LOAD	○	1x 1 mL		●		●	LC-MS compatible peptide reconstitution buffer.	RT
CARTRIDGE		8x					Cartridge with SPE sorbent for cleaning up peptides.	RT
WASTE TUBE		8x					2.0 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

Pre-Requisites

Common lab equipment is required for the sample preparation.

Consumables	Quantity and Description
REACTION TUBE	1.5 mL tubes with low protein retention/binding are recommended (e.g. Eppendorf Protein LoBind® Tubes, catalogue number 0030108116).

Equipment	Quantity and Description
PIPETTE	Standard single-channel pipettes can be used. However, a dispenser or multichannel pipettes are recommended if possible.
THERMOSHAKER	Two devices are recommended to support the different temperatures of the protocol steps. NOTE: We recommend a shaking speed of 1200 rpm for all bead handling steps, but please adjust the shaking speed so that the EN-BEADS are kept in a solution mixed thoroughly and do not splash and form droplets on the lid.
MAGNETIC RACK	For separating the magnetic beads from the supernatant solution (e.g. Invitrogen DynaMag™-2, catalogue number 12321D).
ULTRASONIC BATH	Optional: can be used to resuspend peptides.
CENTRIFUGE	Benchtop centrifuges for 1.5/2.0 mL standard tubes are required for peptide clean-up. Alternatively, positive pressure manifolds can be used. Please consult the ENRICH-iST 96x kit protocol for further information or write to info@preomics.com.
VACUUM CONCENTRATOR	To evaporate volatile buffers from the eluate before LC-MS.

Sample	Quantity and Description
SAMPLE	20 µL mammalian plasma or serum, centrifugated and stored in an anticoagulant. We recommend the use of EDTA anticoagulants and centrifugation at 2000 g. Using a lower or higher input volume is possible, but it might influence the results. Please refer to our FAQ www.preomics.com/faq for further information.

Procedure

Method

Attention: In the iST-BCT box, you will find a protocol for performing the iST-BCT workflow. Please discard it as it is not needed for performing ENRICH-iST. All necessary instructions are given below.

Make sure all reagents are at room temperature when using them. Plasma should be kept frozen until it is needed and should be brought to room temperature and vortexed before pipetting (e. g. 2500 rpm for 20 s).

1. BEADS PREPARATION

1.1. Mix the **EN-BEADS** ◯ vial thoroughly by vortexing until completely resuspended (~20 s).

1.2. Add 25 µL of **EN-BEADS** ◯ to each REACTION TUBE. Make sure the beads stay in suspension during pipetting (vortex when necessary).

1.3. Add 200 µL **EN-WASH** 🟢 to the **EN-BEADS** ◯ in each REACTION TUBE.

1.4. Place REACTION TUBES on a THERMOSHAKER (RT; 1200 rpm; 1 min).

1.5. Place REACTION TUBES on the MAGNETIC RACK. Wait until the beads have formed a pellet. We recommend allowing for 1 min. Carefully remove the supernatant with a pipette and discard it without disturbing the beads pellet.

1.6. Repeat step 1.3. – 1.5. two more times for a total of three washing steps.

2. ENRICH

2.1. Add 80 µL **EN-BIND** 🟠 to each bead pellet in the REACTION TUBES.

2.2. Add 20 µL of SAMPLE to each REACTION TUBE.

2.3. Remove REACTION TUBES from the MAGNETIC RACK and place them on a THERMOSHAKER (30 °C; 1200 rpm; 30 min).

2.4. Place the REACTION TUBES on the MAGNETIC RACK. Wait until the beads have formed a pellet. We recommend allowing for 1 min. Carefully discard the supernatant without disturbing the beads pellet.

2.5. Add 100 µL **EN-BIND** 🟠.

2.6. Remove the REACTION TUBES from the MAGNETIC RACK and place them in a THERMOSHAKER (RT; 1200 rpm; 1 min).

2.7. Place REACTION TUBES on the MAGNETIC RACK. Wait until the beads have formed a pellet. We recommend allowing for 1 min. Carefully discard the supernatant without disturbing the beads pellet.

2.8. Repeat step 2.5. – 2.7. two more times for a total of three washing steps.

3. LYSE

3.1. Add 50 µL **LYSE-BCT** 🟤 to each bead pellet in the REACTION TUBES.

3.2. Remove the REACTION TUBES from the MAGNETIC RACK and place them in a pre-heated THERMOSHAKER (95 °C and 1200 rpm for 10 min). *NOTE1*

3.3. Optional: Quick spin to remove condensation from the lid.

3.4. Allow samples to cool down to RT.

4. DIGEST

4.1. Add 210 µL **RESUSPEND-BCT** to **DIGEST** (1 tube for 4 reactions) and shake (RT; 500 rpm; 10 min). Do not store resuspended DIGEST but use it within one day. Keep it at 2-8 °C if it is not used for several hours.

4.2. Add 50 µL **DIGEST** to each REACTION TUBE.

4.3. Place the REACTION TUBES in a pre-heated THERMOSHAKER (37 °C, 1200 rpm; 1 - 3 h). Digesting for more than 1 h will slightly decrease missed cleavages, but not improve protein identifications substantially.

4.4. Add 100 µL **STOP** to each REACTION TUBE, shake (RT; 1200 rpm; 1 min).

5. PURIFY

5.1. Place CARTRIDGES in WASTE TUBES by using ADAPTERS. Label all tubes.

5.2. Resuspend the beads by pipetting up/down or shaking (RT; 1200 rpm; 1 min). Transfer the samples including the beads to the CARTRIDGES.

5.3. Place CARTRIDGES in a centrifuge and spin (RT; 3800 rcf; 1 min or until flow-through is complete).

5.4. Add 200 µL **WASH 1** to the CARTRIDGES.

5.5. Repeat step 5.3.

5.6. Add 200 µL **WASH 2** to the CARTRIDGES.

5.7. Repeat step 5.3.

5.8. Discard WASTE TUBES. Place CARTRIDGES on top of the COLLECTION TUBES. Label all tubes.

5.9. Add 100 µL **ELUTE** to the CARTRIDGES.

5.10. Spin CARTRIDGES in a centrifuge (RT; 3800 rcf; 1 min or until flow-through is complete).

5.11. Repeat steps 5.9 and 5.10. Collect the flow-through in the same COLLECTION TUBE.

5.12. Discard CARTRIDGES and place COLLECTION TUBES in a VACUUM CONCENTRATOR (RT; until completely dry). *SP*

5.13. Add 15 µL **LC-LOAD** to COLLECTION TUBES and shake (RT, 500 rpm, 5 min). Using 15 µL will typically result in 0.6 – 1.0 mg/mL peptide concentration; adapt volumes to suit your LC-MS setup requirements.

5.14. Optional: Spin COLLECTION TUBES in a CENTRIFUGE to remove particles (maximum rcf recommended by manufacturer, 5 min).

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

NOTE1 The LYSE temperature may be reduced if necessary. This may result in a lower coverage of proteins that need harsh denaturation conditions.

SP - Storage Point: At this point, peptides can be frozen at -80 °C for long-term storage. Storing peptides in STOP buffer for a short period of time is also possible but may slightly reduce protein identifications. Please refer to the FAQ section on www.preomics.com/faq for further information.

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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