PREOMICS

P2-iST Plasma **12x**

P.O.00264; P.O.00269; P.O.00277; P.O.00279

Human EDTA plasma

Introduction

Blood plasma can be a challenging matrix for proteomic analyses due to its complexity and high dynamic range. The P2-iST Plasma kit provides a novel enrichment workflow that enables deep insight into the human blood plasma proteome. Biognosys' well-established P2 particle combined with the proven PreOmics® iST technology offers a fast and robust sample preparation solution that can be easily automated on third-party platforms. For specific recommendations and optimization, visit www.preomics.com/resources or contact info@preomics.com.

Kit Contents

The kit includes all essential components for proteomic sample preparation: protein enrichment, denaturing, reducing, and alkylating agents, enzymes, cartridges, and wash buffers for peptide cleanup. For additional labware needed, please see the "Pre-Requisites" section below.

BOX1:

Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Neutral		
Part 1:								
P2-BIND		1x 5 mL					Facilitates protein binding onto the beads.	2–8°C
P2-BEADS		1x 0.4 mL					Paramagnetic beads that bind plasma proteins.	2–8°C
P2-WASH		1x 10 mL				●	For washing beads after the plasma protein binding step.	2–8°C
Part 2:								
DIGEST-L		1x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND		1x 1 mL				●	For reconstituting lyophilized proteolytic enzymes.	RT
LYSE A		1x 1.4 mL					For denaturing, reducing, and alkylating proteins.	RT
LYSE B		1x 1.4 mL					For denaturing, reducing, and alkylating proteins.	RT
STOP		1x 1.4 mL	●	●			For stopping enzymatic activity.	RT
WASH 1		2x 1 mL	●	●			For removing hydrophobic contaminants.	RT
WASH 2		2x 1 mL		●			For removing hydrophilic contaminants.	RT
ELUTE		2x 1.4 mL	●		●		For eluting peptides from the cartridge.	RT
LC-LOAD		1x 1.4 mL		●			For loading peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		12x					Cartridges with SPE sorbent for peptide purification sealed with silicone mat.	RT
WASTE TUBE		12x					2 mL tube for collecting waste after washing steps.	RT
COLLECTION TUBE		12x					1.5 mL tube for collecting peptides after elution.	RT
ADAPTER		12x					Enables a cartridge to be placed into a tube. It can be used for further experiments.	RT

BOX2: Only available for P.O.00277/P.O.00279 – For more details please read *NOTE1*

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). This recommended plasticware is included with order P.O.00277 / P.O.00279. For additional details, please refer to *NOTE1*

Equipment	Quantity and Description
PIPETTE	Standard single-channel pipettes can be used. However, dispenser or multichannel pipettes are recommended if available. Careful sample handling and pipetting reduce contamination and improve quantification.
HEATING SHAKER	Two heating shakers for multi-well plates are recommended to accommodate the different temperature requirements of the protocol steps. ATTENTION: For the ENRICH part, a shaking speed of 1,000 rpm is recommended for all bead handling steps. Adjust the speed as needed to ensure the P2-BEADS remain thoroughly suspended in the solution and prevent droplet formation on the lid.
MAGNETIC RACK	For separating the magnetic beads from the supernatant solution (e.g., Invitrogen DynaMag™-2, catalogue number 12321D).
CENTRIFUGE	Benchtop centrifuges for 1.5/2 mL standard tubes are required for peptide cleanup.
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
PLASMA	100 µL human EDTA plasma sample, centrifugated and stored in anticoagulant. Centrifugation at 2,000 rcf is recommended. Other anticoagulants or biological fluids may not be compatible. Please contact our technical experts if you plan to test divergent sample types.

Procedure

Method

IMPORTANT: Ensure that all reagents stored at 2–8°C are at room temperature before use. PLASMA SAMPLE should be kept frozen until required. Before pipetting, allow sample to thaw to room temperature and vortex thoroughly to ensure homogeneity (e.g., 2,500 rpm for 20 s). This protocol includes an additional transfer step that requires extra **REACTION TUBES** (24 tubes total). *NOTE1*

1. ENRICH

1.1. Add 75 µL of **P2-BIND** to each REACTION TUBE.
1.2. Add 100 µL of SAMPLE to each REACTION TUBE.
1.3. Mix the **P2-BEADS** tube thoroughly by vortexing until completely resuspended (~20 s).
1.4. Add 25 µL of **P2-BEADS** to each REACTION TUBE. Make sure the beads stay in suspension during pipetting (vortex when necessary).
1.5. Place REACTION TUBES on a pre-heated HEATING SHAKER (30°C; 1,000 rpm; 30 min).
1.6. 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 bead pellet. *NOTE2*
1.7. Remove REACTION TUBES from MAGNETIC RACK and add 200 µL **P2-WASH** to each bead pellet. Carefully pipette up and down to resuspend the beads.
1.8. Transfer bead suspensions into new REACTION TUBES and place them on a HEATING SHAKER (RT; 1000 rpm; 1 min).
1.9. 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 bead pellet. *NOTE2*
1.10. Remove REACTION TUBES from the MAGNETIC RACK and add 200 µL **P2-WASH** to each bead pellet. Carefully pipette up and down to resuspend the beads.
1.11. Place REACTION TUBES on a HEATING SHAKER (RT; 1000 rpm; 1 min).
1.12. 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. *NOTE2*
1.13. Repeat steps 1.10–1.12 one more time for a total of three washing steps.
1.14. After the third washing step 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 bead pellet *NOTE2*

2. LYSE

2.1. The **LYSE** volume needed per sample is 40 µL. Calculate the total volume of **LYSE** required for your experiment. Prepare the calculated **LYSE** volume by mixing equal volumes of **LYSE-A** and **LYSE-B** in a suitable vessel. Briefly vortex the LYSE mixture.
2.2. Add 40 µL **LYSE** to each bead pellet.
2.3. Remove REACTION TUBES from the MAGNETIC RACK and make sure all beads are in suspension (Caution: pipetting up and down causes foaming).
2.4. Place REACTION TUBES on a pre-heated HEATING SHAKER (60°C; 1,300 rpm; 10 min).
2.5. Optional: Quick spin to remove condensation from the lid.
2.6. Allow samples to cool down to room temperature.

3. DIGEST

3.1. Optional: Spin down lyophilized enzyme mix in the **DIGEST-L** tube (RT; max. 300 rcf; 10 sec).

3.2. Add 300 µL **RESUSPEND** to **DIGEST-L** (1 tube for 24 reactions) and shake (RT; 500 rpm; 10 min).

3.3. Add 10 µL **DIGEST-L** to each REACTION TUBE.

3.4. Place REACTION TUBES on a pre-heated HEATING SHAKER (37°C; 1,300 rpm; 60 min).

3.5. Add 60 µL **STOP** to each REACTION TUBE and place them on a HEATING SHAKER (RT; 1,300 rpm; 1 min).

3.6. Place REACTION TUBES on the MAGNETIC RACK. Wait until the beads have formed a pellet. We recommend allowing for 1 min. *Critical* Proceed immediately to Step 4 without delay.

4. PURIFY

4.1. Place **CARTRIDGES** in **WASTE TUBES** by using **ADAPTERS**.

4.2. While still on the MAGNETIC RACK, carefully aspirate the supernatant from the samples and transfer it to the **CARTRIDGES** without disturbing the bead pellet.

4.3. Place **CARTRIDGES** in a CENTRIFUGE and spin (1,000 rcf; 1 min). If needed, adjust time to ensure complete flow-through.

4.4. Add 100 µL **WASH 1** to the **CARTRIDGES** and centrifuge (2,250 rcf; 1 min).

4.5. Add 100 µL **WASH 2** to the **CARTRIDGES** and centrifuge (2,250 rcf; 1 min).

4.6. Discard **WASTE TUBES.** Place **CARTRIDGES** in **COLLECTION TUBES** using **ADAPTERS**.

4.7. Add 75 µL **ELUTE** to the **CARTRIDGES.** Place **CARTRIDGES** in a CENTRIFUGE and spin (1,000 rcf; 1 min). Keep flow-through in the **COLLECTION TUBES**.

4.8. Add further 75 µL **ELUTE** to the **CARTRIDGES** and centrifuge (1,000 rcf; 1 min). Keep flow-through in the same **COLLECTION TUBES**.

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

4.10. Add 20 µL **LC-LOAD** to the wells. Using 20 µL will typically result in 0.2–0.3 µg/µL peptide concentration. Adjust the volume according to specific requirements.

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

4.12. Spin down droplets (RT; 300 rcf; 10 sec).

4.13. Perform a peptide quantification assay. *NOTE3*

4.14. Adjust the peptide concentration according to the LC–MS column loading requirements. It is recommended to centrifuge the samples prior to LC–MS analysis (RT; 2,250 rcf; 10 min).

NOTE1 Two kit versions are available: P.O.00264 / P.O.00269 and P.O.00277 / P.O.00279.
For P.O.00277 / P.O.00279, the recommended plasticware (Eppendorf 1.5 mL Protein LoBind® TUBES) is included. This version is shipped in two boxes:

- BOX 1: P2-iST Plasma Kit
- BOX 2: Required plasticware for the transfer step

Both kit versions contain all materials necessary to perform the P2-iST Plasma workflow manually.
For sample processing on the KingFisher™ system, different plasticware is required. Please refer to the *Quick Start Guide for P2-iST Plasma on KingFisher* for detailed instructions - Contact info@preomics.com.

NOTE2 Bead settling time depends on magnetic strength of the rack and device/tube geometry. Extend settling time if needed. Ensure the pipette tip is positioned at the center of the tube bottom, and aspirate slowly to avoid bead loss.

NOTE3 For accurate adjustment of peptide injection amount, a peptide quantification should be performed. It is recommended using a quantitative fluorometric assay (e.g., Pierce Quantitative Fluorometric Peptide Assay Kit, Thermo Scientific™, catalogue number 23290), with an input of 2 µL peptide sample + 8 µL LC-LOAD.

SP - Storage Point: Dried peptides, prior to reconstitution in LC-LOAD, can 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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