PREOMICS

iST-REG-PSI 192x HT

P.O.00108; P.O.00235

Pelleted cells & precipitated protein

Introduction

Sample preparation is an essential step for bottom-up proteomics. The PreOmics® iST sample preparation kit is designed to assist researchers in achieving the best results with few sample preparation steps and little hands-on time. For sample-specific protocols and optimization visit www.preomics.com/resources or contact info@preomics.com.

Kit Contents

total of two packages

The kit contains everything to perform a complete sample preparation. It includes all chemicals to denature, reduce, and alkylate proteins as well as the enzymes to perform a tryptic digestion and a final peptide cleanup on a positive pressure device. Kit components and the protocol were optimized for the positive pressure device Resolvex® A200 (Tecan).

Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Neutral		
DIGEST		2 vials					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		2x 15 mL	●	●			For stopping enzymatic activity.	RT
WASH 1		2x 100 mL	●	●			For removing hydrophobic contaminants.	RT
WASH 2		2x 100 mL		●			For removing hydrophilic contaminants.	RT
ELUTE		2x 100 mL	●		●		For eluting peptides from the cartridge.	RT
LC-LOAD		2x 25 mL		●			For loading peptides on reversed-phase LC-MS column.	RT
PSI PLATE 96x		2x					96-well plate with SPE sorbent for peptide purification from 1–100 µg protein starting material.	RT

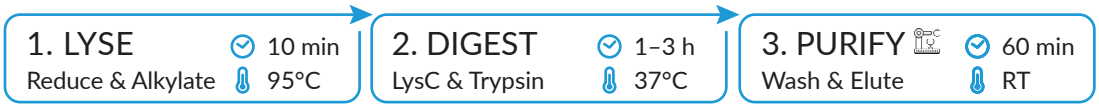
Pre-Requisites

Common lab equipment is required for the sample preparation.

Equipment	Quantity and Description
PIPETTE	Careful sample handling and pipetting reduces contaminations and improves quantification.
SAMPLE	Pelleted cells or precipitated protein (1–100 µg protein starting material; max. 10 µL sample volume). For higher sample volumes, visit our FAQs. For other sample types, contact PreOmics for adapted protocols.
REACTION PLATE	Samples may be handled in any reaction vessel >450 µL, but a 96 deep well plate is recommended (e.g., Eppendorf Deepwell plate 96/500 µL Protein LoBind®, catalogue number EU: 0030504100, US: 951032107).
SEALING MAT	Prevents sample contamination and evaporation (e.g., Eppendorf Sealing Mat, catalogue number EU&US: 0030127978).
WASTE PLATE/ADAPTER	96 deep well plate or a suitable adapter if the integrated waste drain of the positive pressure device is used for sample loading and washing on a positive pressure device.
ELUTE PLATE	96 deep well plate for elution on a positive pressure device (Protein LoBind® plates are recommended to minimize sample loss). Please see specific recommendation for elution plates in the section for Resolvex® A200 (Tecan).
HEATING SHAKER	Two heating shakers for multi-well plates are recommended to support protein denaturation and digestion.

CENTRIFUGE	Optional: Swing-bucket centrifuges for spin down after lysis.
SONICATOR or NUCLEASE	If the sample is viscous, use a sonicator to shear DNA or add nuclease (e.g., Benzonase®) to degrade both DNA and RNA. Visit our FAQ for more information.
POSITIVE PRESSURE DEVICE	This protocol contain all specifications for sample loading, washing, and elution on Resolvex®A200 (Tecan). For other positive pressure devices, please contact info@preomics.com.
VACUUM EVAPORATOR	To evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.

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. LYSE

1.1. Add 50 µL **LYSE** to 1–100 µg of protein sample in a a REACTION PLATE, close plate with a SEALING MAT and place the plate in a pre-heated HEATING SHAKER (95°C; 1,000 rpm; 10 min).

1.2. Optional: Spin down droplets (RT; max. 300 rcf; 10 sec).

1.3. If sample is viscous, use a SONICATOR or add NUCLEASE.

1.4. Allow samples to cool to room temperature.

2. DIGEST

2.1. Add 5 mL **RESUSPEND** to **DIGEST** (1 vial for 96 reactions), invert vial several times (RT; 10 min). *NOTE2*

2.2. Add 50 µL **DIGEST** to each well, close REACTION PLATE with a SEALING MAT and place the plate in a pre-heated HEATING SHAKER (37°C; 500 rpm; 1–3 h).

2.3. Add 100 µL **STOP** to each well (precipitation may occur), shake (RT; 500 rpm; 1 min) and pipette up/down. *SP*

3. PURIFY

3.1. Place the **PSI PLATE 96x** on top of an appropriate WASTE PLATE or ADAPTER and transfer sample to the **PSI PLATE 96x**. *AM*

3.2. Place the **PSI PLATE 96x** on a POSITIVE PRESSURE DEVICE and start the pre-compiled method covering the following steps (3.3–3.12). *AM*

3.3. Apply positive pressure to allow samples to flow through SPE membrane. Adjust time and pressure to ensure complete flow-through. *AM* (see pressure profiles A200_load)

3.4. Add 200 µL **WASH 1** to **PSI PLATE 96x** . *AM*

3.5. Apply positive pressure to allow samples to flow through SPE membrane. Adjust time and pressure to ensure complete flow-through. *AM* (see pressure profiles A200_wash 1)

3.6. Add 200 µL **WASH 2** to **PSI PLATE 96x**. *AM*

3.7. Apply positive pressure to allow samples to flow through SPE membrane. Adjust time and pressure to ensure complete flow-through. *AM* (see pressure profiles A200_wash 2)

3.8. Place **PSI PLATE 96x** on top of an ELUTE PLATE. Label plate and wells.

3.9. Add 100 µL **ELUTE** ● to **PSI PLATE 96x**. *AM*

3.10. Apply positive pressure to allow samples to flow through SPE membrane, keep flow-through in ELUTE PLATE. Adjust time and pressure to ensure complete flow-through. *AM* (see pressure profiles A200_elute 1+2)

3.11. Add 100 µL **ELUTE** ● to **PSI PLATE 96x**. *AM*

3.12. Apply positive pressure to allow samples to flow through SPE membrane, keep flow-through in the same ELUTE PLATE. Adjust time and pressure to ensure complete flow-through. *AM* (see pressure profiles A200_elute 1+2)

3.13. Discard **PSI PLATE 96x** and place ELUTE PLATE in a vacuum evaporator (45°C; until completely dry). *SP*

3.14. 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.

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

3.16. Spin **ELUTE PLATE** in a CENTRIFUGE (RT; maximum rcf recommended by manufacturer; 5-15 min) and transfer the supernatant to a clean plate and avoid touching the bottom of the well during transfer. *NOTE3*

NOTE1 Volumes of buffers can be adjusted according to protein starting amounts. Lysis temperature should be between 60–95°C. Visit our FAQ website for more information.

NOTE2 Resuspended DIGEST can be stored for up to two weeks at 4°C. For longer storage periods, visit our FAQ.

NOTE3 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.

SP - Storage Point: At this point, close the peptide containing plate 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.

AM - Automation method: Kit components as well as the protocol were optimized for the positive pressure device Resolvex® A200 (Tecan). In the following, recommendations for instrument settings, consumables as well as instrument methods and pressure profiles will be provided. Please be aware that these are recommendations and settings may need to be adjusted depending on the instrument.

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.

For our General Terms and Conditions, refer to www.preomics.com.

For trademark information, visit www.preomics.com/legal/trademarks.

Resolvex® A200 (Tecan)

For more information, please visit the Tecan website for the corresponding application note or consult with your Tecan application scientist. For WASH 1, WASH 2, and ELUTE: buffer volumes per vial are optimized for one complete purification run on the Resolvex® A200 platform processing 96 samples. If further runs are envisioned and additional buffers are required, we also provide the iST-REG-PSI Buffer Add-on kit (P.O.00109). The Resolvex® A200 must be configured with a 96-well format positive pressure manifold.

General instrument settings

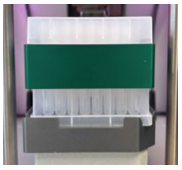
Only critical parameters are listed below. For all other parameters not indicated here, default settings are applied:

IMPORTANT Gas supply	Make sure that the gas supply is set to 80 psi by a regulator. Nitrogen is favored above compressed air to avoid artificial oxidation.
Settings\Pumps\Flush Vol [µLs]	1500
Settings\Pumps\Flush Count	3
Solvents\Other\AntiDrip Volume [µL/w]	50

Work deck and buffer preparation

- ddH2O should be used as "Flush Solvent" and is not provided in the kit.
- WASH 1, WASH 2, and ELUTE are provided in vials that can be directly placed onto the Resolvex® A200 platform. Before starting the purification protocol on the Resolvex® A200, unscrew the buffer vials and replace the lids with suitable lids that have recesses for the buffer lines of the Resolvex® A200. Make sure that the buffer lines reach the bottom of the vials.
- Before starting the protocol, prime all buffer lines that are used in the protocol (WASH 1, WASH 2, ELUTE, ddH2O as “Flush Solvent”) using at least 10 mL of each buffer for priming. Make sure that no air bubbles are visible in the lines. *IMPORTANT* To guarantee optimal purification performance, it is essential to keep the following priming sequence: WASH 1 – WASH 2 – ELUTE – ddH2O.

Recommended consumables and platform setup

Steps	Recommended plastic ware	Set-up on Resolvex® A200 platform (from top to bottom)	
LOAD and WASH (steps 3.1–3.7)	No plasticware required	**96 WELL SPE PLATE** Auto slide spacer BGO Green (Tecan Ref. 30246380) Rise high spacer BGO Red (Tecan Ref. 30168299)	
ELUTE (steps 3.8–3.12)	**ELUTE PLATE:** Eppendorf Deepwell Plate 96/500 µL (Protein LoBind Plate) Reference number EU: 0030504100, US: 951032107	**96 WELL SPE PLATE** Auto slide spacer BGO Green (Tecan Ref. 30246380) **ELUTE PLATE** Adapter barrier nesting (Tecan Ref. 30179631)	

Method

Step	Operation	Solvent	Message	Pressure Profiles
LOAD				
Step 3.3	Flash			A200_load
WASH 1				
Step 3.4	Dispense reagent	200 µL WASH 1		
Step 3.5	Flash			A200_wash 1
WASH 2				
Step 3.6	Dispense reagent	200 µL WASH 2		
Step 3.7	Flash			A200_wash 2
Change plates for elution				
Step 3.8	Message only		"Switch to collection plate"	
ELUTE 1				
Step 3.9	Dispense reagent	100 µL ELUTE		
Step 3.10	Flash			A200_elute 1+2
ELUTE 2				
Step 3.11	Dispense reagent	100 µL ELUTE		
Step 3.12	Flash			A200_elute 1+2

Pressure profiles

The pressure profiles listed below are only recommendations. When setting up the method on your positive-pressure device for the first time—whether for a new sample type or a new application—verify that complete flow-through is achieved and adjust the pressure or processing time as needed. The optimal velocity of the flow-through is one droplet per second.

Important: The *Pressure Range* is set to "High" for all recommended pressure profiles and the pressure is given in [%]. The time is given in [sec] and specifies the total time of the pressure profile.

Example: For the pressure profile A200_load, the pressure is increased from 0% to 8% within 2 sec and then hold for 33 sec (total time: 35 sec]; pressure is then increased to 15% within 2 sec (total time: 37 sec) and hold for 5 secs (total time: 42 sec); pressure is then decreased to 0% within 3 sec (total time: 45 sec) and hold for 15 sec (total time: 60 sec).

A200_load

Time [sec]	Pressure [% of high pressure profile]
0.0	0.00
2.0	8.00
72.0	8.00
75.0	15.00
85.0	15.00
88.0	0.00
100.0	0.00
Total time: 100 sec	

A200_wash 1

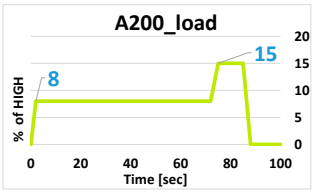
Time [sec]	Pressure [% of high pressure profile]
0.0	0.00
2.0	7.50
72.0	7.50
75.0	15.00
85.0	15.00
88.0	0.00
100.0	0.00
Total time: 100 sec	

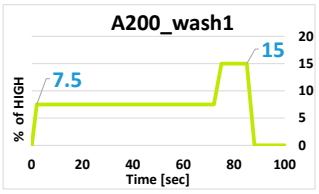
A200_wash 2

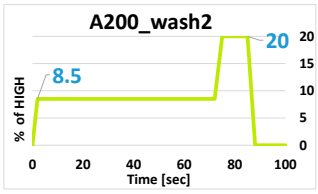
Time [sec]	Pressure [% of high pressure profile]
0.0	0.00
2.0	8.50
72.0	8.50
75.0	20.00
85.0	20.00
88.0	0.00
100.0	0.00
Total time: 100 sec	

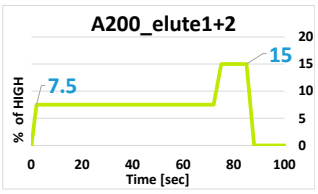
A200_elute 1+2

Time [sec]	Pressure [% of high pressure profile]
0.0	0.00
2.0	7.50
72.0	7.50
75.0	15.00
85.0	15.00
88.0	0.00
100.0	0.00
Total time: 100 sec	









If you need larger buffer volumes of WASH 1, WASH 2, and ELUTE, we also provide the iST-REG-PSI Buffer Add-on kit (P.O.00109) . For more information or order requests, please also visit www.preomics.com or contact order@preomics.com.

For other positive pressure devices (e.g., Hamilton [MPE][2]), please contact info@preomics.com.