PREOMICS

iST-REG-PSI 96HT **192x**

Pelleted cells & precipitated protein

Introduction

Sample preparation is an essential steps 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/downloads or contact info@preomics.com.

Kit Contents

per package,
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 devices Resolvex® A200 (Tecan) and [MPE]^2 (Hamilton).

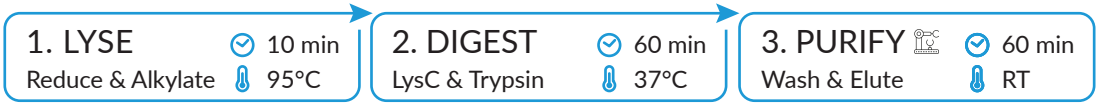
Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Volatile		
DIGEST	red	2 vials					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND	yellow	1x 20 mL				●	Reconstitutes lyophilized proteolytic enzymes.	RT
LYSE	brown	1x 20 mL			●		Denatures, reduces and alkylates proteins.	RT
STOP	black	2x 15 mL	●	●		●	Stops the enzymatic activity.	RT
LC-LOAD	white	2x 25 mL		●		●	Loads peptides on reversed-phase LC-MS column.	RT
WASH 1	blue	2x 100 mL	●	●		●	Cleans peptides from hydrophobic contaminants.	RT
WASH 2	green	2x 100 mL		●		●	Cleans peptides from hydrophilic contaminants.	RT
ELUTE	pink	2x 100 mL	●		●	●	Elutes the peptides from the SPE-PLATE.	RT
96 WELL SPE-PLATE		2x					96-well plate for 1-100 µg protein starting material per well.	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. For other sample types contact PreOmics for adapted protocols.
96 WELL SKIRTED PLATE	96 well skirted plate for lysis and digestion (Protein LoBind plates are recommended to minimize sample loss).
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).
HEATING BLOCK	Two MTP plate heaters are recommended to support protein denaturation and digestion.
SONICATOR	If the sample contains DNA, shear it by sonication (e.g. Diagenode Bioruptor®).
POSITIVE PRESSURE DEVICE	Positive pressure device, e.g. the Resolvex®A200 (Tecan) or the [MPE]^2 (Hamilton), for sample loading, washing and elution.
VACUUM EVAPORATOR	Vacuum manifolds evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.

Procedure



Method

1. LYSE

1.1. Add 50 µL **LYSE** to 1-100 µg of protein sample in a 96 WELL SKIRTED PLATE, place it in a HEATING BLOCK (95°C; 1,000 rpm; 10 min). *NOTE1*

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

1.3. If the sample contains DNA, shear it in a SONICATOR (10 cycles; 30 sec ON/OFF). Let sample cool down to RT.

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 sample and place it in a pre-heated HEATING BLOCK (37°C; 500 rpm; 1-3 hours). *NOTE3*

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

3. PURIFY

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

3.2. Place the **96 WELL SPE-PLATE** 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/[MPE]²_load)

3.4. Add 200 µL **WASH 1** to **96 WELL SPE-PLATE**. *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/[MPE]²_wash 1)

3.6. Add 200 µL **WASH 2** to **96 WELL SPE-PLATE**. *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/[MPE]²_wash 2)

3.8. Place **96 WELL SPE-PLATE** on top of an ELUTE PLATE. Label plate and wells.

3.9. Add 100 µL **ELUTE** to **96 WELL SPE-PLATE**. *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/[MPE]²_elute 1)

3.11. Add 100 µL **ELUTE** to **96 WELL SPE-PLATE**. *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/[MPE]²_elute 2)

3.13. Discard **96 WELL SPE-PLATE** and place ELUTE PLATE in a vacuum evaporator (45°C; until completely dry).

3.14. Add **LC-LOAD** to ELUTE PLATE. Aim for 1 g/L concentration (e.g., 100 µL to 100 µg protein starting material).

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

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: www.preomics.com/faq.

NOTE2 Lyophilized enzyme mix is stable for 9 months at -20°C. Resuspended enzyme mix can be stored for 4 weeks at -20°C.

NOTE3 During the digestion, close the 96 WELL SKIRTED PLATE with a silicon mat.

SP - Storage Point: At this point, close the peptide containing plate/tube. Peptides can be frozen at -20°C. Storage of peptides should not exceed 2 weeks at -20°C. For extended storage, finish the protocol and store at -80°C.

AM - Automation method: Kit components as well as the protocol were optimized for the positive pressure devices Resolvex®A200 (Tecan) and [MPE]² (Hamilton). 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.

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 were 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.

General instrument settings

Only critical parameters are listed below. For all other parameters not indicated here, default settings were 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
Settings\Pump\AntiDrip Volume [µLw]	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 set-up

Steps	Recommended plastic ware	Set-up on Resolvex® A200 platform (from top to bottom)	
LOAD and WASH (steps 3.1 - 3.7)	No plastic ware required	**96 WELL SPE PLATE** Auto slide spacer BGO Gold (Tecan) Rise high spacer BGO Red (Tecan)	
ELUTE (steps 3.8 - 3.12)	**ELUTE PLATE:** Eppendorf Deepwell Plate 96/500 µl (Protein LoBind Plate) Reference number: 0030 504.100	**96 WELL SPE PLATE** Auto slide spacer BGO Gold (Tecan) **ELUTE PLATE** Adapter barrier nesting 96 well SPE (Tecan)	

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 you set up the method on your positive pressure device the first time, for a new sample type, or a new application, please check that complete flow-through of the samples is ensured and adjust pressure or time if necessary. 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

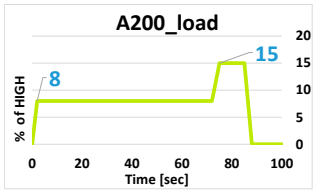
Time [sec]	Pressure [% of high pressure profile]
0.0	0.00
2.0	7.50
72.0	7.50
75.0	15.00
85.0	15.00
88.0	0.00
100.0	0.00
Total time: 100 sec	

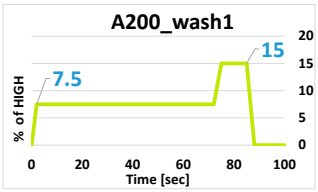
A200_wash 2

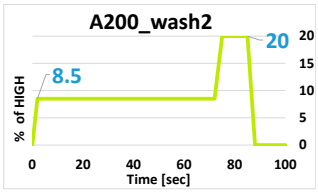
Time [sec]	Pressure [% of high pressure profile]
0.0	0.00
2.0	8.50
72.0	8.50
75.0	20.00
85.0	20.00
88.0	0.00
100.0	0.00
Total time: 100 sec	

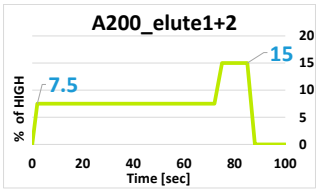
A200_elute 1+2

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

[MPE]² (Hamilton)

For detailed information concerning general instrument settings, work deck preparation or recommended consumables, please visit the Hamilton website for the corresponding application note or consult with your Hamilton application scientist.

Pressure profiles

The pressure profiles listed below are only recommendations. When you set up the method on your positive pressure device the first time, for a new sample type, or a new application, please check that complete flow through of the samples is ensured and adjust pressure or time if necessary. The optimal velocity of the flow-through is one droplet per second.

Important: The pressure is given in [psi]. The time is given in [sec] and specifies the duration.

Example: For the pressure profile [MPE]²_load, the pressure is set to 5.00 psi for 20 sec (total time: 20 sec); the pressure is then increased to 7.00 psi and hold for 120 sec (total time: 140 sec); the pressure is then increased to 10.00 psi and hold for 20 sec (total time: 160 sec).

[MPE]²_load

Time [sec]	Pressure [psi]
20.0	5.00
120.0	7.00
20.0	10.00
Total time: 160 sec	

[MPE]²_wash 1

Time [sec]	Pressure [psi]
40.0	5.00
20.0	7.00
Total time: 60 sec	

[MPE]²_wash 2

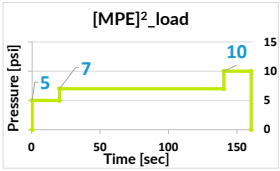
Time [sec]	Pressure [psi]
40.0	5.00
20.0	15.00
Total time: 60 sec	

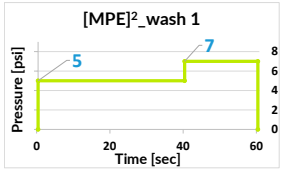
[MPE]²_elute 1

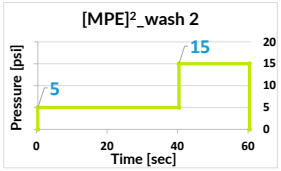
Time [sec]	Pressure [psi]
60.0	4.00
Total time: 60 sec	

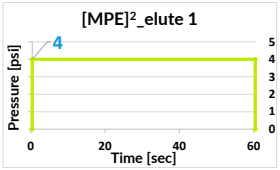
[MPE]²_elute 2

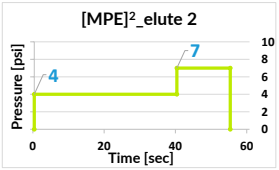
Time [sec]	Pressure [psi]
40.0	4.00
15.0	7.00
Total time: 55 sec	











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