PREOMICS

iST Sample Preparation Kit **8x**

Agarose Immunoprecipitation Samples

Introduction

The PreOmics sample preparation kit is compatible with immunoprecipitation (IP) and co-immunoprecipitation (co-IP) samples. This protocol is compatible with IP/co-IP samples processed with agarose beads. For a specific protocol compatible with IP/co-IP samples processed with magnetic beads, please contact us or visit our website at www.preomics.com.

Kit Contents

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.

Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Volatile		
DIGEST		2x					Trypsin/LysC mix to digest proteins.	-20°C
RESUSPEND		1x 2 mL				●	Reconstitutes lyophilized proteolytic enzymes.	RT
LYSE		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		●		●	Loads peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		8x					Cartridge for 1 to 100 µg protein starting material.	RT
WASTE		8x					2.0 mL tube for collecting waste after washing steps.	RT
COLLECTION		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.

Equipment	Quantity and Description
ULTRACENTRIFUGE TUBE	1.5/2.0 mL reaction tube for LYSE and DIGEST steps
PIPETTE	Careful sample handling and pipetting reduces contaminations and improves quantification.
SAMPLE	IP/co-IP samples processed with Agarose beads.
HEATING BLOCK	Two heating blocks are recommended to support protein denaturation and digestion.
CENTRIFUGE	1.5/2.0 mL reaction tube centrifuges are required for 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

Perform your IP/co-IP using your own protocol. Stop after washing the agarose beads/affinity matrix.

Remove your wash buffer completely and directly proceed with step 1.1. of the PreOmics protocol. *Critical Note*

1 LYSE

1.1. Add 50 µL **LYSE** to the washed beads in a microcentrifuge tube, place in a HEATING BLOCK (60°C; 1,000 rpm; 10min). *NOTE1*

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

2. DIGEST

2.1. Add 210 µL **RESUSPEND** to **DIGEST** (1 tube for 4 reactions), shake (RT; 500 rpm; 10 min), pipette up/down.

2.2. Add 50 µL **DIGEST** to sample in the microcentrifuge tube and place in a pre-heated HEATING BLOCK (37°C; 500 rpm; 1-3 hours).

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

2.4 Centrifuge beads (RT; 2,500 rcf; 2 min).

3. PURIFY

3.1. Use **ADAPTER** to place **CARTRIDGE** in **WASTE** tube. Label all tubes.

3.2. Transfer the complete supernatant (combined LYSE/DIGEST/STOP buffers) to **CARTRIDGE**. Discard beads.

3.3. Spin **CARTRIDGE** in a CENTRIFUGE (3,800 rcf; 1-3 min). If needed, adjust values to ensure complete flow-through.

3.4. Add 200 µL **WASH 1** to **CARTRIDGE**, repeat step 3.3.

3.5. Add 200 µL **WASH 2** to **CARTRIDGE**, repeat step 3.3. *SP*

3.6. Use **ADAPTER** to place **CARTRIDGE** in a fresh **COLLECTION** tube. Label all tubes.

3.7. Add 100 µL **ELUTE** to **CARTRIDGE**, repeat step 3.1., keep flow-through in **COLLECTION** tube.

3.8. Repeat step 3.8., keep flow-through in the same **COLLECTION** tube.

3.9. Discard **CARTRIDGE** and place **COLLECTION** tube in a vacuum evaporator (45°C; until completely dry).

3.10. Add **LC-LOAD** to **COLLECTION** tube. Aim for 1 g/L concentration (e.g. 100 µL to 100 µg protein starting material).

3.11. Sonicate **COLLECTION** tube in an ULTRASONIC BATH (5 min) or shake (RT; 500 rpm; 5 min). *SP*

Critical Note Make sure that the very last wash step after the IP/co-IP does not include any detergents. Ideally use your IP wash buffer without detergents or blocking protein e.g. PBS. Contact us at info@preomics.com if you have any further questions

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.

SP - Storage Point: At this point, close the peptide containing tube or **CARTRIDGE** using silicon lid. Peptides can be frozen at -20°C. Storage of peptides should not exceed two weeks at -20°C. For extended storage, finish the protocol and store at -80°C.

Data analysis

Consider the following as fixed modifications in your database search:

MODIFICATION	DESCRIPTION	COMPOSITION	SPECIFICITY	MASS	UNIMOD #
ALKYKATION	Carbamidomethyl on cysteine	C_2H_3NO	[C]	+57Da	4

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