Sample Preparation Kit
PROTOCOL – Agarose Immunoprecipitation Samples

COMPATIBILITY

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. For IP/co-IP of GFP-fusion proteins, we highly recommend to use the iST GFP-Trap® Kit, exclusively distributed by our partner ChromoTek GmbH.

INTRODUCTION

Sample preparation is one of the essential steps of bottom-up proteomics. The PreOmics sample preparation kit is designed to assist you achieving best results with few sample preparation steps and little hands-on time.

KIT CONTENTS

The iST kit provides a streamlined solution for reliable sample preparation compatible with IP/co-IP samples. It includes all chemicals to denature, reduce & alkylate proteins as well as the enzymes to perform a tryptic digest and a final cleanup.

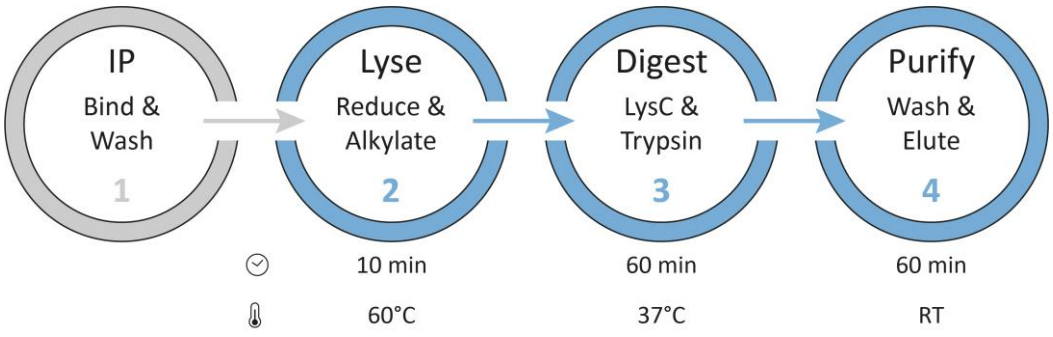
COMPONENT	CAP	QUANTITY	BUFFER PROPERTIES Organic	Acidic	Basic	Volatile	DESCRIPTION	STORAGE
DIGEST	red	2x					Enzyme *Trypsin*-mix to digest proteins.	-20 °C
RESUSPEND	yellow	1x 1 mL				✓	Protease reconstitution buffer for enzymes.	RT
LYSE	brown	1x 1 mL			✓		Denature, reduce and alkylate proteins.	RT
STOP	black	1x 1 mL	✓	✓		✓	Stop the enzymatic activity.	RT
WASH 1	blue	1x 2 mL	✓	✓		✓	Clean up peptides from hydrophobic contaminants.	RT
WASH 2	green	1x 2 mL		✓		✓	Clean up peptides from hydrophilic contaminants.	RT
ELUTE	violet	1x 2 mL	✓		✓	✓	Elute the peptides from the cartridge.	RT
LC-LOAD	white	1x 1 mL		✓		✓	Load peptides on reversed-phase LC-MS column.	RT
CARTRIDGE		8x					Cartridge for 1 to 100 µg protein starting material.	RT
WASTE		8x					Tube for collecting waste after washing steps.	RT
COLLECTION		8x					Tube for collecting peptides after elution.	RT
ADAPTER		8x					Enables placing a cartridge into a tube.	RT
CAP		8x					Cap to optionally close the cartridge's bottom.	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.
HEATING BLOCK	Heating shakers are recommended to help protein denaturation and during digestion.
CENTRIFUGE	Eppendorf tube centrifuges are necessary for loading, washing and elution.
SPEED-VAC	Vacuum manifolds evaporate volatile buffers from the eluate before LC-MS.
ULTRASONIC BATH	Optional: can be used to resuspend peptides.

PROCEDURE



Material: *IP/co-IP samples processed with agarose beads*
Quantity: *100 µg protein starting material*

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PROTOCOL

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 and place it in a pre-heated HEATING BLOCK (60 °C; 1,000 rpm; 10 min).

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

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

3. PURIFY

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

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

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

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

3.5. Add 200 µL **WASH 1** ● to **CARTRIDGE**, repeat step 3.1., discard flow-through.

3.6. Add 200 µL **WASH 2** ● to **CARTRIDGE**, repeat step 3.1., discard flow-through. *SP*

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

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

3.9. Repeat step 3.5., keep flow-through in the same **COLLECTION** tube.

3.10. Remove **CARTRIDGE** and place **COLLECTION** tube in a SPEED-VAC (45 °C; until completely dry).

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

3.12. 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. Best would be your lysis buffer without detergents. Contact us at info@preomics.com if you have any further questions.

SP - Storage Point: At this point, close the peptide containing tube or **CARTRIDGE** (use a **CAP** for bottom). 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.

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]	+57 Da	4

DISCLAIMER

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