

Sample Preparation Kit

PROTOCOL – Immunoprecipitated proteins



COMPATIBILITY

The PreOmics sample preparation kit is compatible with immunoprecipitation of EGFP-fusion protein samples processed with the ChromoTek GFP-Trap®_M (magnetic beads, gtm) and the GFP-Trap®_MA (magnetic agarose beads, gtmA) produced by ChromoTek GmbH (Martinsried, Germany, for details visit www.chromotek.com).

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. For sample-specific protocols and optimization contact us or visit our website at www.preomics.com.

KIT CONTENTS

The kit contains all you need 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 digest and a final clean up.

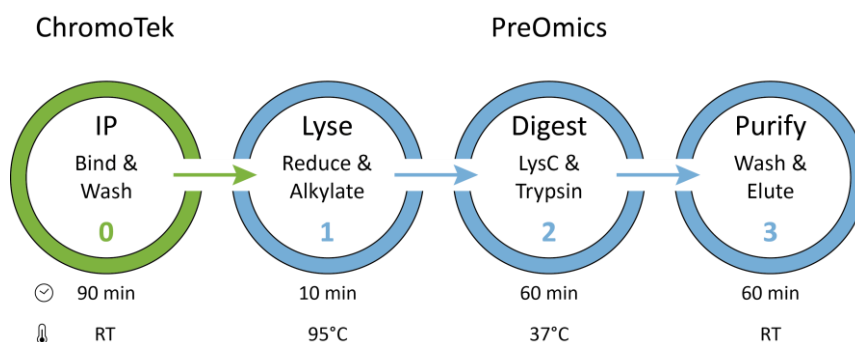
COMPONENT	CAP	QUANTITY	BUFFER PROPERTIES				DESCRIPTION	STORAGE
			Organic	Acidic	Basic	Volatile		
DIGEST	red	2x					Enzyme <i>Trypsin</i> -mix to digest proteins.	-20 °C
RESUSPEND	yellow	1x 1 mL				✓	Protease reconstitution buffer for enzymes.	RT
LYSE	brown	1x 1 mL				✓	Denature, reduce and alkylate 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.
SAMPLE	Lyophilized protein or pelleted cells. For other materials ask us for adapted protocols.
HEATING BLOCK	Heating shakers are recommended to help protein denaturation and during digestion.
CENTRIFUGE	Eppendorf tube centrifuges are necessary for loading, washing and elution.
SONICATOR	If you have DNA in your sample, shear it by sonication (e.g. Diagenode Bioruptor®).
SPEED-VAC	Vacuum manifolds evaporate volatile buffers from the eluate before LC-MS.

PROCEDURE



Material: Immunoprecipitated protein
 Quantity: 100 µg protein starting material

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PROTOCOL

Follow the protocol for immunoprecipitation of GFP-fusion proteins using either the GFP-Trap®_M or the GFP-Trap®_MA by ChromoTek GmbH. Stop after washing the beads/affinity matrix (step 7 in the ChromoTek protocol).

1. LYSE

- 1.1. Add 50 µL **LYSE** ● to the washed beads and place it in a pre-heated HEATING BLOCK (95 °C; 1,000 rpm; 10 min).
- 1.2. Optional: Spin down droplets (RT; max. 300 rcf; 10 sec).
- 1.3. Transfer the complete slurry (beads and LYSE buffer) to **CARTRIDGE** and cool down (RT).
- 1.4. Use **ADAPTER** to place **CARTRIDGE** in **WASTE** tube. Label all tubes.

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 **CARTRIDGE** and place it in a pre-heated HEATING BLOCK (37 °C; 500 rpm; 1-3 hours).
- 2.3. Add 100 µL **STOP** ● to **CARTRIDGE** (precipitation may happen), shake (RT; 500 rpm; 1 min), pipette up/down. *SP*

3. PURIFY

- 3.1. Spin **CARTRIDGE** in a CENTRIFUGE (3,800 rcf; 1-3 min). If needed, adjust values to ensure complete flow-through.
- 3.2. Add 200 µL **WASH 1** ● to **CARTRIDGE**, repeat step 3.1., discard flow-through.
- 3.3. Add 200 µL **WASH 2** ● to **CARTRIDGE**, repeat step 3.1., discard flow-through. *SP*
- 3.4. Use **ADAPTER** to place **CARTRIDGE** in a fresh **COLLECTION** tube. Label all tubes.
- 3.5. Add 100 µL **ELUTE** ● to **CARTRIDGE**, repeat step 3.1., keep flow-through in **COLLECTION** tube.
- 3.6. Repeat step 3.5., keep flow-through in the same **COLLECTION** tube.
- 3.7. Remove **CARTRIDGE** and place **COLLECTION** tube in a SPEED-VAC (45 °C; until completely dry).
- 3.8. Add **LC-LOAD** ○ to **COLLECTION** tube. Aim for 1 g/L concentration (e.g. 100 µL to 100 µg protein starting material).
- 3.9. Sonicate **COLLECTION** tube in a SONICATOR (max. power; 5 min). *SP*

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 ₂ H ₃ NO	[C]	+57 Da	4

DISCLAIMER

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Material: Immunoprecipitated protein
Quantity: 100 µg protein starting material