PREOMICS

SP3-iST Add-on Kit **96x**

Biological samples and purified proteins (diluted, dirty, pretreated)

Introduction

The PreOmics SP3-iST Add-on kit is a perfect complement to our iST sample processing technology and is designed as an upstream protein handling step to concentrate and purify proteins after efficient sample lysis and denaturation. The SP3-iST workflow is characterized by its high versatility and is compatible with a wide range of sample matrices and buffer conditions. For further information including buffer compatibility visit www.preomics.com/downloads or contact info@preomics.com.

Kit Contents

The kit supplements the PreOmics iST sample preparation kits and is compatible with all kits from the iST, iST-BCT and iST-NHS series. The kit contains all buffers and chemicals to efficiently lyse and denature samples and to perform an upstream protein binding step utilizing the SP3 technology.

Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Volatile		
SP3 LYSE (2-fold)*	brown	1x 8 mL		●			Denatures and reduces proteins.	RT
SP3 BEADS	white	1x 10 mL					For protein binding.	RT
RESUSPEND	yellow	1x 20 mL				●	For dilution of SP3 lysis buffer and SP3 beads washing and resuspension.	RT
SP3 BIND	orange	1x 20 mL	●			●	Facilitates protein binding onto beads.	RT
SP3 WASH	blue	2x 25 mL	●			●	Cleans proteins on beads.	RT

* The SP3 LYSE buffer might become slightly viscous in cold conditions. Please make sure that it becomes liquid again before usage by shaking and gentle heating (approx. 30 °C). This does not affect buffer performance.

Pre-requisites

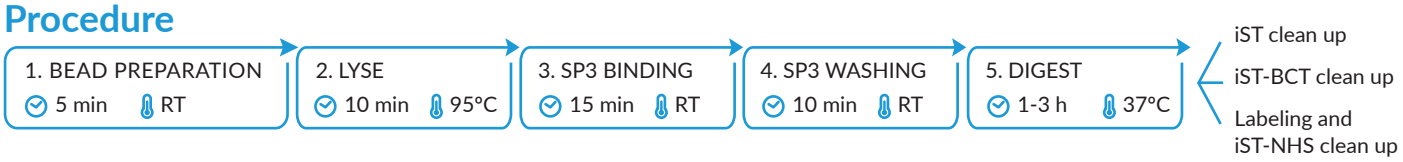
Common lab equipment is required for the sample preparation. Additional lab equipment might be required for the iST | iST-BCT | iST-NHS workflow (see corresponding instruction manual).

Consumables	Quantity and Description
DEEP WELL PLATE	For SP3 BINDING and SP3 WASHING, samples may be handled in any reaction vessel >=500 µL, but a 96x deep well plate is recommended (e.g. Eppendorf Deepwell plate 96/500 µL Protein LoBind®, Cat. Nr. 0030504100).
SEALING MAT	Prevents sample contamination and evaporation (e.g. Eppendorf Sealing Mat, Cat.-Nr. 0030127978).
ADDITIONAL PLASTIC VESSELS	For the LYSE step, tubes or plates are required that are suitable for the heating step (95 °C, 1000 rpm for 10 min) and if applicable, sonication.
BUFFERS and ENZYMES from the iST | iST-BCT | iST-NHS kit	For efficient protein alkylation, digestion and peptide clean-up, buffers and enzymes from the iST/ iST-BCT/iST-NHS kit are required. LYSE ● | LYSE-BCT ● | LYSE-NHS ● ; RESUSPEND ● | RESUSPEND-BCT ● ; DIGEST ● ; STOP ● ; WASH 1 ● ; WASH 2 ● ; ELUTE ● ; LC-LOAD ○

Equipment	Quantity and Description
PIPETTE	Careful sample handling and pipetting reduces contaminations and improves quantification.
MAGNETIC SEPARATOR	For separating the magnetic beads from the supernatant solution, use a magnetic separator (e.g. Alpaqua Magnum FLX® for 96-well plates, Cat.-Nr. A000400).
THERMOSHAKER	For bead preparation and washing (RT, 1200 rpm), protein denaturation (95 °C, 1000 rpm) and digestion (37 °C, 1200 rpm). NOTE: We recommend a shaking speed of 1200 rpm for all bead handling steps, but please adjust the shaking speed so that the SP3 BEADS are kept in solution, mixed thoroughly and do not splash or form droplets on the sealing mat.
SONICATOR	If the sample contains DNA, shear it by sonication (e.g. Diagenode Bioruptor®).

Sample	Quantity and Description
SAMPLE	1-100 µg protein starting material in a maximum of 50 µL starting volume. Samples can be previously lysed in your own lysis buffer.

Procedure



Method

1. BEAD PREPARATION

1.1. Mix the **SP3 BEADS** ◯ vial thoroughly by vortexing and make sure that beads are completely resuspended (~15 sec).

1.2. Transfer the appropriate volume of **SP3 BEADS** ◯ according to the sample protein amount to each well of a DEEP WELL PLATE.

Protein input amount	Required volume of SP3 BEADS
1 – 10 µg	10 µL
11 – 50 µg	50 µL
51 – 100 µg	100 µL

1.3. Place the DEEP WELL PLATE on a MAGNETIC SEPARATOR and wait until **SP3 BEADS** ◯ have formed a pellet. Carefully discard supernatant without disturbing the **SP3 BEADS** ◯.

1.4. Wash beads by adding 50 µL of **RESUSPEND** 🟡 to the **SP3 BEADS** ◯.

1.5. Place the SEALING MAT on the DEEP WELL PLATE.

1.6. Place the DEEP WELL PLATE on a THERMOSHAKER (RT; 1200 rpm; 1 min).

1.7. Place the DEEP WELL PLATE on a MAGNETIC SEPARATOR and wait until **SP3 BEADS** ◯ have formed a pellet. Carefully discard supernatant without disturbing **SP3 BEADS** ◯.

1.8. Add 20 µL of **RESUSPEND** 🟡 to the **SP3 BEADS** ◯, place SEALING MAT on the DEEP WELL PLATE and remove the DEEP WELL PLATE from the MAGNETIC SEPARATOR.

1.9. Keep the **SP3 BEADS** ◯ in **RESUSPEND** 🟡 until SP3 BINDING (step 3.1.).

2. LYSE

2.1. Add 50 µL **SP3 LYSE** 🟤 to 1-100 µg of protein sample in an appropriate PLASTIC VESSEL and make up to 100 µL with **RESUSPEND** 🟡. *NOTE1*; *NOTE2*

2.2. Place sample in a pre-heated THERMOSHAKER (95 °C; 1000 rpm; 10 min).

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

2.4. If the sample contains DNA, shear it by SONICATION (10 cycles; 30 sec ON/OFF).

2.5. Let sample cool down to RT.

3. SP3 BINDING

3.1. Transfer sample to the DEEP WELL PLATE with prepared **SP3 BEADS** ◯.

3.2. Add 120 µL of **SP3 BIND** 🟠.

3.3. Place a SEALING MAT on the DEEP WELL PLATE.

3.4. For SP3 binding, place the DEEP WELL PLATE on a THERMOSHAKER (RT; 1200 rpm: 15 min).

4. SP3 WASHING

4.1. Place the DEEP WELL PLATE on a MAGNETIC SEPARATOR and wait until **SP3 BEADS** ◯ have formed a pellet. Carefully discard supernatant without disturbing the **SP3 BEADS** ◯.

4.2. Add 150 µL **SP3 WASH** 🔵 to the sample.

4.3. Place a SEALING MAT on the DEEP WELL PLATE.

4.4. Place the DEEP WELL PLATE on a THERMOSHAKER (RT; 1200 rpm; 1 min).

4.5. Place the DEEP WELL PLATE on a MAGNETIC SEPARATOR and wait until **SP3 BEADS** have formed a pellet. Carefully discard supernatant without disturbing the **SP3 BEADS**.

4.6. Repeat steps 4.2. - 4.5. two more times to wash proteins bound to beads three times in total.

4.7. Make sure that **SP3 WASH** is completely removed from sample.

5. DIGEST

5.1. Add 50 µL LYSE or LYSE-BCT or LYSE-NHS to sample. Do not mix the beads by pipetting up and down.

5.2. Prepare DIGEST according to the instructions in the iST, iST-BCT or iST-NHS protocol.

5.3. Add 50 µL resuspended DIGEST to sample. Make sure that the **SP3 BEADS** are covered with liquid and, if necessary, gently move the beads into the solution by pipetting along the plate wall without touching the beads with the pipette tip or gently shake the sample.

5.4. Place SEALING MAT on the DEEP WELL PLATE.

5.5. Place sample in a pre-heated HEATING BLOCK (37 °C; 1200 rpm; 1-3 hours). *NOTE3 for SP3 coupled to iST-BCT*; *NOTE4*

6. CONTINUE WITH iST KITS

6.1. Continue according to the appropriate kit protocol:

For iST kit:

-Add 100 µL **STOP**, mix thoroughly.

-Transfer sample including the **SP3 BEADS** to the CARTRIDGE and continue with step ´3. PURIFY´ of iST protocol.

For iST-BCT kit:

- Add 100 µL **STOP**, mix thoroughly.

- Transfer sample including the **SP3 BEADS** to the CARTRIDGE and continue with step ´3. PURIFY´ of iST-BCT protocol.

For iST-NHS kit:

- Place sample on the MAGNETIC SEPARATOR and transfer supernatant to new PLASTICWARE and continue with step ´3. LABEL´.

NOTE1 Samples can be previously lysed in your own lysis buffer (maximum sample volume in lysis buffer is 50 µL). Follow the protocol and do not skip any steps. For buffer compatibility, refer to the FAQs www.preomics.com/faq or contact info@preomics.com.

NOTE2 Samples can be lysed in the tube/plate of your choice that is suitable for the heating step (95 °C, 1000 rpm for 10 min and sonication. For efficient lysis of tissue or deparaffinized tissue samples, add 40-50 mg glass beads to sample. Add 50 µL **SP3 LYSE** to sample and make up to 100 µL with **RESUSPEND**. Shear sample in SONICATOR (10 cycles; 30 sec ON/OFF). Place sample in HEATING BLOCK (95 °C; 1000-1400 rpm; 10 min). For tougher tissue like heart or muscle, repeat sonication and boiling steps one more time.

NOTE3 for SP3 coupled to iST-BCT For efficient on-beads digestion and optimal peptide recovery, we recommend performing digestion for 3 hours (37 °C, 1000 – 1400 rpm).

NOTE4 Optional, an aqueous elution step can be performed. Adjust the pH of sample to pH 8-9 with NaOH solution (added volume should not exceed 10 µL) and shake sample (RT; 1400 rpm; 5 min). IMPORTANT: After the addition of **STOP**, make sure that the pH of the sample is acidic (pH 3-4).