PREOMICS

SP3-iST (8rxn) Add-on Kit **8x**

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.

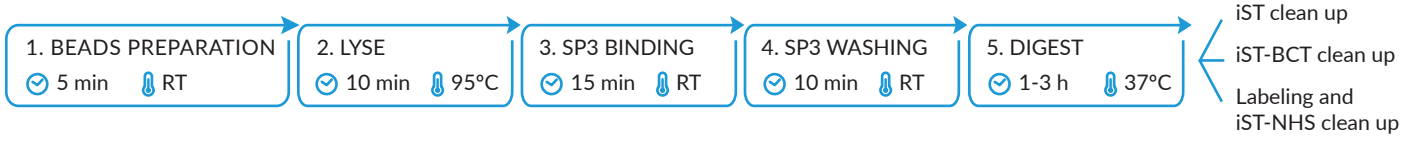
Component	Cap	Quantity	Buffer Properties				Description	Storage
			Organic	Acidic	Basic	Volatile		
SP3 LYSE (2-fold)*	brown	1x 1 mL		●			Denatures and reduces proteins.	RT
SP3 BEADS	white	1x 1 mL					For protein binding.	RT
RESUSPEND	yellow	1x 2 mL				●	For dilution of SP3 lysis buffer and SP3 beads washing and resuspension.	RT
SP3 BIND	orange	1x 2 mL	●			●	Facilitates protein binding onto beads.	RT
SP3 WASH	blue	3x 2 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).

Equipment	Quantity and Description
PIPETTE	Careful sample handling and pipetting reduces contaminations and improves quantification.
TUBES or PLATE	1.5/2 mL tubes or deep well plate are required. Low protein binding tubes or plates are recommended to minimize sample loss.
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.
MAGNETIC SEPARATOR	For magnetic beads handling.
HEATING SHAKER	For protein denaturation (95 °C, 1000 rpm) and digestion (37 °C, 1000-1400 rpm)
SONICATOR	If the sample contains DNA, shear it by sonication (e.g. Diagenode Bioruptor®).
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 ○



Method

1. BEADS PREPARATION

1.1. Mix the **SP3 BEADS** ◯ tube thoroughly by vortexing and make sure that beads are completely resuspended and do not stick to tube wall or cap.

1.2. To minimize sample handling steps, beads for several reactions can be prepared in one tube. Transfer the appropriate volume of **SP3 BEADS** ◯ according to the number of samples and the sample protein concentration into a new TUBE.

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

*E.g. for 3 samples containing 50 µg protein, transfer 3x 50 µL of **SP3 BEADS** ◯ into a new tube.*

1.3. Place TUBE on MAGNETIC SEPARATOR and wait until **SP3 BEADS** have formed a pellet. Carefully discard supernatant without disturbing the beads.

1.4. Wash **SP3 BEADS** ◯ by adding the following amount of **RESUSPEND** 🟡 to the TUBE, pipette up/down 3 to 4 times away from the MAGNETIC SEPARATOR:

Protein input amount	Required volume of RESUSPEND
1 – 10 µg	10 µL
11 – 50 µg	25 µL
51 – 100 µg	50 µL

*E.g. when **SP3 BEADS** ◯ for 3 samples containing 50 µg protein are prepared in one TUBE, 75 µL of **RESUSPEND** 🟡 are used.*

1.5. Place TUBE on MAGNETIC SEPARATOR, and wait until **SP3 BEADS** ◯ have formed a pellet. Carefully discard supernatant without disturbing beads.

1.6. Resuspend **SP3 BEADS** ◯ by adding 20 µL **RESUSPEND** 🟡 per reaction (regardless of protein input amount) to the TUBE, pipette up/down 3 to 4 times away from the MAGNETIC SEPARATOR.

*E.g. when **SP3 BEADS** ◯ for 3 samples are prepared in one TUBE, 60 µL **RESUSPEND** 🟡 is used.*

1.7. 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 TUBE and make up to 100 µL with **RESUSPEND** 🟡. *NOTE1*; *NOTE2*

2.2. Place sample in a HEATING BLOCK (95 °C; 1000-1400 rpm; 10 min).

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

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

3. SP3 BINDING

3.1. Mix prepared **SP3 BEADS** ◯ from step 1.7 thoroughly by pipetting up/down. Add 20 µL of prepared **SP3 BEADS** ◯ to sample.

3.2. Add 120 µL **SP3 BIND** 🟠 to sample; carefully shake sample. Do not flip or invert to avoid beads sticking to the tube walls.

3.3. Incubate sample at 1000-1400 rpm and RT for 15 min.

4. SP3 WASHING

4.1. Place sample on MAGNETIC SEPARATOR and carefully discard supernatant without disturbing the beads.

4.2. Add 150 µL **SP3 WASH** to sample, pipette up/down 3 to 4 times away from the MAGNETIC SEPARATOR.

4.3. Place sample on MAGNETIC SEPARATOR and wait until **SP3 BEADS** have formed a pellet. Carefully discard supernatant without disturbing the beads.

4.4. Repeat steps 4.2 and 4.3 two more times to wash proteins bound to beads three times in total.

4.5. 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. Gently move the beads that are not covered by liquid into the solution by pipetting along the tube wall without touching the beads with the pipette tip or gently shake the sample. Carefully mix by pipetting up and down the sample, try to avoid beads sticking to the pipette tip.

5.4. Place sample in a pre-heated HEATING BLOCK (37 °C; 1000-1400 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 CARTRIDGE and continue with step ´3. PURIFY´ of iST instruction manual.

For iST-BCT kit:

- Add 100 µL STOP, mix thoroughly.

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

For iST-NHS kit:

- Place sample on the MAGNETIC SEPARATOR and transfer supernatant to new TUBE 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 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 (10 cycles; 30 sec ON/OFF). For tougher tissue like heart or muscle, repeat sonication and boiling 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).