PREOMICS

Frequently asked questions (FAQs) – Version 2.0

PREOMICS

INTRODUCTION

PreOmics - Setting the Standard for Protein Analysis

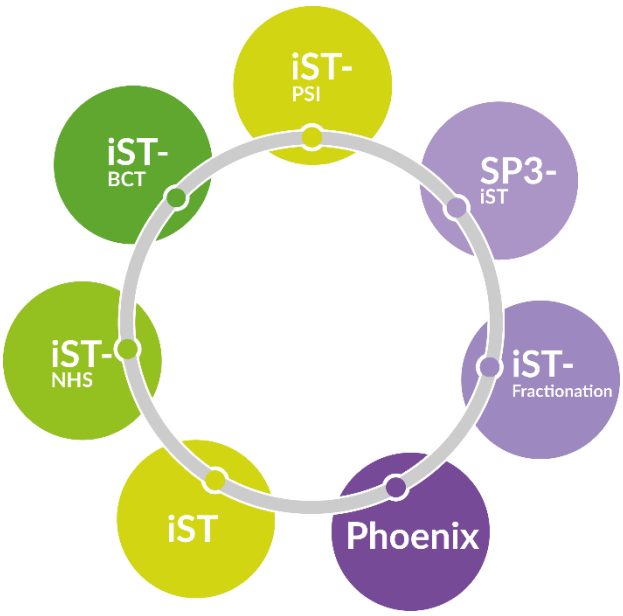
Sample preparation for proteins, peptides and proteomic analysis by MS made easy.

Proteomic methodologies have traditionally been carried out by experienced scientific personnel as it involved complex protocols which needed optimization for each sample type, the methods were time-consuming and routinely achieving reliable and reproducible data was challenging. PreOmics addresses these limitations with the iST technology, enabling robust and simple-to-use sample preparation with a significant time advantage compared to other commonly employed methods.

The iST and iST-BCT kits are compatible with label-free and metabolic labeling (e.g. SILAC) experiments. The iST-NHS kits are compatible with chemical labeling (e.g. iTRAQ™ or TMT™) experiments. The iST and iST-NHS kits contain all reagents and consumables needed to process whole cells, tissues, body fluids, precipitated proteins and other sample types. The iST-BCT is specifically designed for plasma and serum samples. The iST kit is also available in iST-PSI format, a 96 fixed well plate suitable for automation.

The iST-Fractionation Add-on kit provides a three step fractionation providing a sensible compromise between lab and MS time and the proteome depth. The SP3-iST Add-on kit allows the use of harsh lysis and denaturation reagents, extending the range of samples that can be prepared using the iST technology.

The PHOENIX kit is a specialized SPE (solid phase extraction) peptide cleanup kit and the input material is a solution containing peptides. The PHOENIX offers an easy-to-use solution to clean up peptide mixtures and efficiently removes detergents, polymers, salts, lipids and more for reliable LC-MS analyses.



For more information or to request MSDS, please contact us at info@preomics.com

PREOMICS

FREQUENTLY ASKED QUESTIONS

1. SAMPLE PREPARATION

1.1. Is my own lysis buffer compatible for use with the PreOmics' kits?

We recommend that you use the appropriate LYSE buffer provided with the PreOmics' iST, iST-NHS or iST-BCT kits. If you want to use your own lysis buffer, please use the SP3-iST kit. Please refer to the following table or contact us (info@preomics.com) to discuss the compatibility of your lysis buffer with the standard iST, iST-NHS and iST-BCT kits:

Lysis buffer	PreOmics compatible?	Max. vol. lysis buffer	Considerations
PBS	Yes	25 µL	dilute with equal volume of appropriate 2X concentrated LYSE reagent
RIPA (max. 0.1% SDS, 1% SDC, 1%Triton x-100)	Yes	25 µL	dilute with equal volume of appropriate 2X concentrated LYSE reagent
Urea (max. 2M; no thiourea)	Yes	25 µL	dilute with equal volume of appropriate 2X concentrated LYSE reagent
Thiourea	No	-	perform protein precipitation
High salt (e.g. > 0.5M NaCl)	No	-	perform protein precipitation

1.2. What is the maximum starting volume of sample I can process with the PreOmics kits?

Sample volume (liquid or solid, e.g. cell pellets) should not exceed 10 µL when combined with the appropriate LYSE buffer. For sample volumes between 11-25 µL, use the 2-fold concentrated lysis buffers, which can be purchased upon request. The 2X lysis buffers are mixed 1:1 with samples ranging from 11-25 µL, e.g. 11 µL sample + 11 µL 2X LYSE, all downstream buffer volumes stay the same. If your sample volume exceeds 25 µL, perform protein precipitation before processing with the standard PreOmics' workflows.

1.3. How do I perform protein precipitation?

Several different protocols for protein precipitation exist. We recommend acetone precipitation:

a. Transfer protein lysate (not more than 300 µL) to a clean 2 mL microcentrifuge tube.

→ in case you have a larger sample volume, use either a 5 or 15 mL tube

b. Add ice-cold acetone (-20°C) to your sample

→ add at least 4-fold more acetone than sample, e.g. 300 µL sample + 1.2 mL acetone

c. Mix briefly

d. Incubate for one hour at -20°C

e. Centrifuge at 4°C for 15 min at 13,000 rpm

f. Carefully discard supernatant, make sure not to disturb the pellet

g. Air-dry the pellet for 5-10 min

h. Continue with appropriate PreOmics protocol adding the LYSE

→ you can also freeze the pellet after air-drying at -20°C or -80°C until needed

1.4. Can I add protease inhibitors to my sample?

Although protease inhibitors are in general compatible with our iST protocols, we recommend not to add additional exogeneous proteins in order to not contaminate your sample.

1.5. Can I use mechanical force to help lysis in combination with the PreOmics' kits?

Many different mechanical force methods can be employed such as traditional bead milling, liquid nitrogen grinding, or commercial systems from various vendors (e.g. Bertin Instruments, Covaris, Hielscher, MP Biomedicals). These methods can aid lysis efficiency for samples including cells, yeast or tissues. We recommend the Bioruptor® Pico system (Diagenode), that allows direct placement of our iST and iST-NHS CARTRIDGES (please note iST-BCT and PHOENIX CARTRIDGES are not suitable).

Samples such as biological fluids do not require additional mechanical force disruption.

1.6. What are the minimum and maximum protein amounts for the PreOmics' kits?

Highly reproducible results are achieved with samples containing total protein ranging from 1 µg to 100 µg. For low input information please see 1.9.

1.7. Which protein quantification methods are compatible with the PreOmics' kits?

Most classical assays are compatible with our PreOmics' iST, iST-NHS and iST-BCT LYSE buffers. We recommend the BCA assay or the tryptophan quantification method. Some assays require dilution with distilled water to achieve the best results shown below:

BCA: none	microBCA: 1:100 with PBS	Bradford: 1:4
Coomassie: 1:20	Lowry: 1:4	Tryptophan: none

➔ BCA assay is NOT compatible with SP3-LYSE, please see 8.6 for more information.

1.8. What is the maximum volume I can load on the PreOmics' CARTRIDGES?

The maximum volume is 250 µL.

1.9. Should I adjust the volumes of the buffers with different protein starting amounts?

We recommend using the buffer volumes indicated in the protocol. You can adjust the volume of LYSE and DIGEST to your starting amounts. For less than 20 µg protein starting material use the following volumes: 10 µL LYSE; 10 µL DIGEST; keep the other buffers as indicated in the protocol. Accordingly, you may adjust the volumes for chemical labeling and quenching as recommended by the label manufacturer.

1.10. Which lysis temperature should I use?

Perform the lysis/denaturation step at 95°C. Use lower temperatures only for temperature-sensitive samples such as immunoprecipitations. PreOmics have tested lower temperatures down to 60°C and do not see any differences in parameters assessed (e.g. IDs, alkylation rates).

1.11. Can I use other enzymes for the protein digestion?

The PreOmics' kits come with a lyophilized enzyme mix consisting of LysC and trypsin, we do not supply other enzyme combinations.

1.12. How long should I digest my sample for?

The digestion depends on your sample type and input material (see table below). Please note our recommendations to lower the enzyme amount when working with low input samples (<20 µg protein starting material, see 1.9).

Sample type	Digestion time
Precipitated proteins	1-3 hrs
Cell lines	1-3 hrs
Biological fluids	1-3 hrs
Tissues (mammalian, plants)	3 hrs

Although not recommended, you can also digest your samples overnight (~18 hours). While this will reduce the missed cleavage rate even further, it will come at the cost of higher unspecific cleavages and much longer processing time of the overall workflow.

1.13. Are the PreOmics' workflows compatible with enrichment of phosphorylation sites?

While our protocols are in general compatible with IP samples (modified protocols can be found to download on our website), the required input amounts for global phosphorylation enrichment experiments (~500 µg) usually exceed the peptide binding capacity of our CARTRIDGES.

1.14. How can I automate my sample processing?

The PreOmics' PreON is a dedicated sample preparation platform suitable for 12 or 16 samples, see https://www.preomics.com/preon and section 6 for more information. PreOmics is working with liquid handling systems providers and application notes are available at https://www.preomics.com/ or we are happy to provide technical assistance to transfer the PreOmics methodology onto your existing platform if appropriate.

1.15. Are the PreOmics' kit compatible with absolute quantification?

All of the PreOmics kits are compatible with absolute quantification strategies. For absolute quantification employing isotopically-labeled protein standards, please add the respective absolute standard (e.g. DIGESTIF, PSAQ, SILAC-PrEST) together with your sample to the appropriate LYSE buffer ensuring the total protein does not exceed 100 µg and proceed with the protocol accordingly.

For absolute quantification employing isotopically-labeled peptide standards with the PreOmics kits, please introduce the respective standard (e.g. AQUA, QconCAT) before adding the STOP buffer.

1.16. How do I assess the peptide recovery rate after the complete workflow?

The best way to assess peptide recovery rates is by employing absolute quantification strategies (see 1.15). Our peptide recovery is typically >80% for 1 - 100 µg protein starting material.

1.17. What kind of quality control does PreOmics provide for the kits?

Production of PreOmics' kits is ISO 9001:2015 certified. Quality control measures include incoming goods control, polymer leaching tests for plasticware, LC/MS-based quality control for parameters including alkylation rate or missed cleavages on a standardized sample. Upon request, PreOmics can provide a certificate of analysis (CoA) to our clients.

1.18. What are the differences between 4x, 8x, 12x, 96x and 192x PreOmics' kits?

Our kits are provided in pack sizes according to the number of samples that can be processed:

iST kits

- 4 reactions: test kits for first time users
- 8 reactions: small kits for initial experiments
- 96 reactions: medium kits with flexibility to process either up to 96 samples at once or multiple smaller batches, as all buffers and enzymes are supplied in 2 mL tubes
- 192 reactions: large kit suitable for liquid handling platforms, supplied in 20 mL vials for convenient transfer into liquid handling reservoirs

iST-REG-PSI kits

- 192 reactions: large kit suitable for liquid handling platforms, supplied in 20 mL vials for convenient transfer into liquid handling reservoirs, includes 2 fixed well plates for the PURIFY step.

iST-NHS kits

- 4 reactions: test kits for first time users
- 12 reactions: small kits for initial experiments
- 96 reactions: medium kits with flexibility to process either up to 96 samples at once or multiple smaller batches, as all buffers and enzymes are supplied in 2 mL tubes
- 192 reactions: large kit suitable for liquid handling platforms, supplied in 20 mL vials for convenient transfer into liquid handling reservoirs

iST-BCT kits

- 4 reactions: test kits for first time users
- 8 reactions: small kits for initial experiments
- 96 reactions: medium kits with flexibility to process either up to 96 samples at once or multiple smaller batches, as all buffers and enzymes are supplied in 2 mL tubes

Phoenix kits

- 4 reactions: test kits for first time users
- 96 reactions: medium kits with flexibility to process either up to 96 samples at once or multiple smaller batches, as all buffers supplied in 2 mL tubes

Kit variant	Buffer volumes provided	4 rxn	8rxn	12 rxn	96 rxn	192 rxn
iST	in 2 mL tubes	yes	yes	-	yes	-
iST	in 20 mL vials	-	-	-	-	yes
iST-REG-PSI	in 20 mL vials	-	-	-	-	yes
iST-NHS	in 2 mL tubes	yes	-	yes	yes	yes
iST-NHS	in 20 mL vials	-	-	-	-	-
Phoenix	in 20 mL tubes	yes	-	-	yes	-

1.19. Are the PreOmics' kits compatible with peptide fractionation?

Yes, all our kits are compatible with downstream peptide fractionation workflows. PreOmics has a dedicated iST-Fractionation Add-on kit or alternatively after peptides are dried completely, resuspend the peptides either in LC-LOAD or a resuspension buffer of your choice as input buffer for further peptide fractionation methods.

1.20. When should I use the iST-BCT kit?

The iST-BCT kit has been designed specifically for use with biological fluids plasma and serum samples and for analysis of monoclonal antibodies, therapeutic proteins and host cell proteins (HCPs). The LYSE-BCT provides enhanced reduction and alkylation efficiency and all reagents are optimized to minimize artificial modifications such as oxidation or deamidation.

1.21. What is the difference between iST and iST-BCT kits?

The LYSE-BCT and RESUSPEND-BCT reagent composition has been modified with reduction agent, detergent and pH being optimized to give improved performance. The iST-BCT CARTRIDGE is NOT suitable for sonication and is identical to the PHOENIX CARTRIDGE.

1.22. How can I minimize artificial modifications when using iST-BCT.

For even lower oxidation and deamidation rates and increased alkylation rate, reduce the temperature to 80°C and incubate for 10-20 minutes.

1.23. Do you have to process the samples on the CARTRIDGES provided in the PreOmics' kits?

You can perform the LYSE and DIGEST steps in an appropriate plate or microcentrifuge tube until the addition of the STOP reagent. The total volume should then be transferred to the CARTRIDGE for the purify steps.

2. SAMPLE TYPE RECOMMENDATIONS

2.1. How much raw material do I need for the PreOmics' kits?

Protein content varies considerably across distinct biological input material. Different cell lines, strains, tissues, tissue regions, biological fluids and the sample storage conditions can affect the protein concentration. We recommend carrying out a protein concentration assay of the sample after the lysis step (see 1.7). A short overview of raw material amounts is given in the table below.

We have a database with processing recommendations for >100 species and sample matrices. Please contact us at info@preomics for further details on your specific sample of interest.

Material	**Starting amount**	**Protein amount**
Mammalian cell line (e.g. HeLa)	6E5 cells	100 µg
Yeast (*S.cerevisae*)	OD_{600}=0.6	100 µg
Bacteria (*E.Coli*)	OD_{600}=0.5	100 µg
Immunoprecipitation	1 mL slurry	10 - 400 µg
Blood / Serum / Plasma (*H.sapiens*)	2 µL	100 µg
Mammalian tissue	1 mm^3	100 µg
Plant tissue (*A.thaliana*): shoot/root wet weight	50 mg / 100 mg	100 µg / 100 µg

2.2. How do I process non-depleted plasma/serum samples?

Non-depleted human plasma and serum samples have a high protein concentration, commonly around 50 µg/µL. For processing of non-depleted plasma / serum for non-labeled the iST-BCT kit should be used, for labeled workflows use the iST-NHS kit. Mix 2 µL plasma/serum with 50 µL LYSE-BCT / LYSE-NHS buffer and continue with the regular iST-BCT / iST-NHS protocol.

2.3. How do I process depleted plasma / serum samples?

Depending on the depletion process used, the resulting depleted plasma / serum sample may contain high concentrations of salts and/or have a large volume, making the samples incompatible for direct processing with PreOmics' kits. We suggest performing protein precipitation (see 1.3) and using the resulting pellet as input material for the iST-BCT / iST-NHS protocol.

2.4. How do I process CSF samples?

CSF samples have a wide reported concentration range, therefore PreOmics suggest performing a protein quantification assay to determine the concentration prior to processing (see 1.7). For highly concentrated CSF samples, follow the recommendations

for non-depleted plasma/serum (see 2.2) For diluted CSF samples (volume larger than 25 µL), perform protein precipitation (see 1.3) and continue with the regular PreOmics' protocol. Both iST and iST-BCT are suitable for unlabeled workflows, iST- NHS should be used for labeled workflows.

2.5. How do I process urine samples?

Protein concentrations in urine samples vary substantially, we recommend either concentrating or precipitating the protein (see 1.3) before processing with the PreOmics' kits. As a rule of thumb, 10-100 mL human urine corresponds to ~100 µg protein, which needs to be concentrated down to 100 µL. Specifically for urine preparations, PreOmics provides an extra WASH0 buffer to effectively remove bilirubin contaminations, please review the protocols at https://www.preomics.com/resources.

2.6. How do I process saliva samples?

Protein concentrations in saliva samples vary widely, a rough estimate is about 10 µg/µL. Either collect ~10 µL saliva by spitting into a microcentrifuge tube or perform mouth swab and place the swap in 50-100 µL LYSE / LYSE-NHS buffer to fully cover the swap. Keep all other buffer volumes as indicated in the regular protocols.

2.7. How do I process adherent cell culture samples?

If extracellular matrix or transmembrane proteins are not of interest, you can use cell culture-grade trypsin to detach the adherent cells, wash them once with PBS and then store the cell pellet fraction as input for our regular protocols. Alternatively, if extracellular matrix or transmembrane are of interest and affected by partial trypsin digestion, add 50-100 µL of our LYSE / LYSE-NHS buffer directly to the cells and scrape them off. Collect the scraped material and heat to 95°C for 10 min before continuing with the regular iST / iST-NHS protocol.

2.8. How do I process mammalian tissue samples?

Mammalian tissue samples are more difficult to lyse and require some kind of mechanical force disruption in the presence of our PreOmics' LYSE buffers (see 1.5.). Addition of glass beads in combination with ultrasonication enhances the efficiency of tissue homogenization and cell lysis, or use the SP3-iST kit (Application notes can be found at https://www.preomics.com/resources).

2.9. How do I process FFPE samples?

Processing of FFPE samples requires de-paraffinization of the FFPE punches/slides. In addition, deparaffinized samples require a longer heat treatment for efficient decrosslinking: increase the initial heating step from 10 to 60 min at 95°C. More details can be found in the FFPE protocol or use the SP3-iST kit (protocols available at https://www.preomics.com/resources).

2.10. How do I process plant tissue samples?

Plant tissues can be processed with our iST and iST-NHS kits but require cryogenic grinding or other means of mechanical force disruption initially. Specifically for plant preparations, PreOmics provides an extra WASH0 buffer to effectively remove secondary metabolite contamination, the protocol can be found at

https://www.preomics.com/resources.

2.11. How do I process immunoprecipitation samples?

Enrichment of proteins via IP or co-IP strategies is compatible with our iST and iST-NHS technologies. Depending on the type of bead material used, the sample transfer to our cartridges happens either directly after the IP (transfer of proteins; magnetic beads) or after the digestion (transfer of peptides; agarose beads). For further information, please have a look at our two workflow recommendations available on the protocol tab at https://www.preomics.com/resources

2.12. How do I process bacteria / yeast / algae / diatoms?

Model organisms can be entirely processed with our iST or iST-NHS kits. We recommend mechanical force disruption in the presence of our lysis buffers to effectively disrupt tissues/lyse cells. For processing of algae and diatoms, our WASH0 buffer removes secondary metabolites (provided upon request).

3. CHEMICAL LABELING (iTRAQ™, TMT™)

3.1. What is the difference between the iST and the iST-NHS kits?

The lysis buffer in the iST-NHS kit, called LYSE-NHS, does not contain primary amines and therefore does not interfere with chemical labeling. The LYSE-NHS contains a distinct alkylation agent. Please consider the following as fixed modification in your database search: Specific cysteine modification (C6H11NO), specificity [C], mass shift +113.084 Da

3.2. How can I improve the labeling efficiency when performing chemical labeling in combination with theiST-NHS kits?

Chemical labeling experiments require very high peptide labeling efficiencies (>98%) for proper quantification. When struggling with lower labeling efficiencies, please see the following points:

- Make sure that the sample input material (e.g. cell pellet) is not contaminated with residual buffers or cell culture media containing primary amines that interfere with chemical labeling.
- Use fresh labeling reagents to achieve the highest labeling efficiency. Resuspended labeling agent, which is not used throughout the experiment, should not be stored longer than two weeks at -20°C. Resuspended labeling reagent will hydrolyze over time leading to lower labeling efficiency.
- Use TMT at a label to peptide ratio of 4:1, i.e. 400 µg of TMT label per 100 µg of peptides. Higher ratios will slightly increase the labeling efficiency but commonly reduce peptide identification rates.
- Use an acetonitrile (ACN) concentration of at least 30% during the labeling reaction, i.e. 50 µL LYSE + 50 µL DIGEST + 42 µL of labeling reagent in dry ACN. Lower amounts of ACN will quickly hydrolyze the labeling agent. If you have resuspended the labeling reagent in a smaller ACN volume, add some dry ACN to the solution in order to achieve a final concentration of 30% ACN. Take the volume of the cell pellet and residual buffer/cell culture media into account for the final ACN concentration.

3.3. When should I mix channels after the chemical labeling?

There are two options for channel mixing:

a. Perform the workflow until addition of STOP buffer, then mix channels accordingly. The pooled sample can be split equally on different CARTRIDGES (binding capacity of 100 µg per CARTRIDGE).
b. Perform the workflow until end of vacuum concentrator step. Resuspend individual channels in LC- LOAD and then pool channels accordingly.

We advise measuring the peptide concentration of each channel for optimal channel pooling results.

3.4. Should I perform the chemical labeling step on the cartridge or in the tube?

Perform the labeling in a 1.5 mL tube and only to transfer the labeled peptides to our CARTRIDGES for the final peptide cleanup.

4. POSITIVE PRESSURE PROCESSING

4.1. What is the difference between the iST and iST-REG-PSI HT 192x kits?

The iST-PSI kit contains two fixed well clean-up plates for the purification step on a positive pressure unit. The iST kit clean-up kit has an “array” plate format allowing the individual cartridges to be removed and processed in microcentrifuge tubes using adapters (not included but available for order separately).

4.2. My automation platform has some dead volumes, will there be enough buffers?

Additional buffers for the clean-up steps can be ordered separately if needed. P.O.00109 iST-REG- PSI buffer add-on contains 2 x 100 mL of WASH1, WASH2 and ELUTE.

4.3. How can I process the iST-REG-PSI plates?

The iST-REG-PSI plates have been designed to work optimally when used with positive pressure however they can also be processed by centrifugation.

5. PEPTIDE CLEANUP

5.1. Which sample types require additional wash steps?

Most peptide samples can be cleaned up with the washing buffers in the PreOmics' kits. However, for certain samples such as urine or plant tissues, our regular iST or iST-NHS protocols can be extended with one additional washing buffer called "WASH0" to remove metabolites. When preparing peptide samples with high levels of contamination, e.g. sugars, fat, polymers or high concentrations of detergents, we recommend to use our PHOENIX peptide cleanup kit instead which contains an additional wash step with "WASHX".

5.2. What are the differences between the PreOmics' washing buffers?

Washing buffer	Organic	Acidic	Basic	Volatile	Sample types
WASH0	yes	yes	-	yes	Urine, plants
WASHX	yes	yes	-	yes	Lipids, polymers, detergents
WASH1	yes	yes	-	yes	Hydrophobic contaminations
WASH2	-	yes	-	yes	Hydrophilic contaminations

5.3. How do I elute my samples from the 96-well CARTRIDGE adapter plates?

You can either stack the 96-well CARTRIDGE adapter plate on top of the provided collection plate, or you can stack it on top of standardized autosampler vials.

5.4. How long do I need to concentrate my samples in the vacuum concentrator?

Concentrate at 45°C until the sample is dry and no residual ELUTE buffer is left. This usually takes about 30 min. Depending on your sample, peptide ions might accumulate at the top layer and thus interfere with efficient evaporation. To overcome this, tap the sample briefly to mix the eluate and then continue to concentrate in the vacuum concentrator.

5.5. Can I concentrate PreOmics' eluates together with samples eluted from C18 columns in the same vacuum concentrator?

Since our ELUTE buffer has a basic pH and C18 eluates have an acidic pH, do not place them in the same vacuum concentrator as this can damage the instrument.

5.6. Which peptide quantification methods can I use after processing my samples?

Peptide quantification should be done in our LC-LOAD buffer and not in the ELUTE buffer. We recommend quantitative colorimetric peptide assays.

5.7. How do I resuspend dried peptides in the 96-well plate?

After the vacuum concentrator step, you can add our LC-LOAD buffer to each well and shake in a horizontal plate shaker (500 rpm, 5 min).

5.8. How should I store resuspended peptide samples after processing them?

Storage of peptides should not exceed two weeks at -20°C. For long-term storage, keep them at -80°C.

6. PHOENIX KITS

6.1. Are the CARTRIDGES from all the PreOmics kits the same?

CARTRIDGES in the iST and iST-NHS kits are the same. CARTRIDGES in the iST-BCT and PHOENIX kits are of different composition and have a slightly lower affinity for hydrophobic species.

6.2. How do I load my peptide samples on the PHOENIX CARTRIDGES?

It is essential to acidify the peptides, otherwise your sample will not bind to the CARTRIDGE. Mix your peptides 1:1 with the provided STOP buffer and load everything on the CARTRIDGE. Spin at 3,800 rcf for 1-3 min to load the sample completely.

7. PEPTIDE FRACTIONATION

7.1. What is in the iST-Fractionation Add-on kit?

The iST-Fractionation Add-on kit contains three buffers, please note it does NOT contain the CARTRIDGE or LC- LOAD reagent, therefore this kit should ALWAYS be used in conjunction with one of the iST family of kits.

7.2. Is the iST-Fractionation Add-on kit compatible with the iST-BCT kit and Phoenix clean-up kit?

Yes, the iST-Fractionation Add-on kit is compatible with all of the PreOmics iST family of kits.

7.3. How much LC-LOAD should I use for each fraction?

Add LC-LOAD to COLLECTION tubes 1-3, typically we suggest that you aim for 1 g/L concentration remembering that the starting protein concentration will have been fractionated into 3 (e.g. 90 µL to 90 µg protein starting material equates to 30 µL per tube).

7.4. Are the PreOmics' kits compatible with other peptide fractionation protocols?

Yes, all our kits are compatible with downstream peptide fractionation workflows. After peptides are dried completely, resuspend the peptides either in LC-LOAD or a resuspension buffer of your choice as input buffer for further peptide fractionation methods.

8. SP3-iST ADD-ON KITS

8.1. What is in the SP3-iST Add-on kit?

The SP3-iST Add-on kit contains SP3 beads and reagents for bead preparation, non-biased protein binding, lysis and washing. The kit must be used in conjunction with one of the iST, iST-NHS or iST-BCT kits.

8.2. Which protein extraction buffers are compatible with the SP3-iST Add-on kit?

Reagent name or type	**Tested concentration range**	**Considerations for performing SP3 in the described conditions**
Detergents	0-20%	Detergents tested in the listed concentration range include SDS, Triton X-100, NP-40, Tween 20, deoxycholate, CHAPS, and RapiGest. We recommend keeping the total detergent concentration in the range of 0–10% (wt/vol or vol/vol depending on the detergents used)
Chaotropes	0-8 M	Chaotropes tested in the listed concentration range include urea (up to 8 M), guanidinium hydrochloride, and isothiocyanate (up to4 M). High concentrations of chaotropes in the presence of the solvent used for binding can disrupt the interactions between SP3 beads and the proteins. Therefore, we recommend testing SP3 with your desired lysis solution formulation before further application
Salts	0-1 M	A wide range of salts have been tested, and we recommend keeping the final salt concentration in the lysate <1 M when using solvents for binding. High salt concentrations in the presence of binding solvent can disrupt the ability of proteins to efficiently localize on the SP3 bead surface. The critical concentration is going to depend on the identity and properties of the salt in question
Solvents	0-50%	Solvents tested in the listed concentration range include acetonitrile, acetone, isopropanol, ethanol, trifluoroethanol, and xylene. We recommend keeping the final solvent concentration in the lysate before binding <25% (vol/vol). If high solvent concentrations are present, the amount of ethanol added during SP3 can be scaled to achieve the desired final solvent concentration (e.g., 50% (vol/vol) final)

Hughes, C.S., Moggridge, S., Müller, T. et al. Single-pot, solid-phase-enhanced sample preparation for proteomics experiments. Nat Protoc 14, 68–85 (2019). https://doi.org/10.1038/s41596-018-0082-x

8.3. How do I use my own extraction buffer with the SP3-iST Add-on kit?

Prepare your samples in your own extraction buffer (maximum volume 50 µL) and use the SP3 protocol as given, ensuring in step 2.1 that you add 50 µL of SP3 LYSE and make up to 100 µL with RESUSPEND if needed.

8.4. Do I have to use my own extraction buffer or can I just use SP3 LYSE?

You do not have to use a combination of buffers as the protocol will work successfully using SP3 LYSE alone. Add 50 µL of SP3 LYSE to your sample and make up to 100 µL with RESUSPEND.

8.5. Does the SP3 LYSE contain detergents, reducing and alkylation agents?

The SP3 LYSE buffer contains detergent and reduction agents needed. The alkylation reagent is added in the appropriate kit LYSE reagent.

8.6. Is the SP3 LYSE buffer compatible with protein determination assays?

The SP3 LYSE is not compatible with the BCA assay, we recommend starting with your usual extraction buffer and protein assay, if you do not have a preferred assay then we suggest the Detergent Compatible Bradford Assay from ThermoFisher.

8.7. How do I know what SP3 bead volume to use?

The bead volume is calculated from the number of samples being prepared and the protein concentration of those samples.

For each sample starting protein concentration see the SP3 volume below:

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.

8.8. How do I prepare tissue or FFPE samples with the SP3-iST kit?

We recommend homogenizing the sample in a sonicator with glass beads. Add 40-50 mg glass beads and 50 µL SP3 LYSE to sample and make up to 100 µL with RESUSPEND. Sonicate for at least 10 cycles; 30 sec ON/OFF. Then heat for at least 10 minutes at 95 °C with shaking if possible at 1000- 1400 rpm. For tougher tissue like heart or muscle, repeat sonication and heating step a second time.

8.9. Can we digest in solution rather than on-bead?

Yes, an aqueous elution step can be performed and may be useful when using the iST-BCT kit. 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), then place the sample on the magnetic separator and remove the supernatant to a clean tube for the DIGEST step. IMPORTANT: After the addition of STOP, make sure that the pH of the sample is acidic (pH 3-4).

9. PreON PLATFORM

9.1. What are the pre-installation requirements?

We offer a full pre-installation requirement documentation upon request. Briefly, the PreON operates at: 100–240 V AC, 50/60 Hz, 650 VA. PreON dimensions are (WxDxH): 65 cm (25.6 in.) x 62 cm (24.4 in.) x 86 cm (34.0 in.) with a weight of 71.5 kg (157.6 lbs).

9.2. Which sample types can be processed on the PreON?

The PreON can process protein pellets, intact cells, body fluids and tissue lysates.

9.3. How many samples can I process on the PreON per day?

The sample throughput is 12 samples per run with the option to execute multiple runs per day (16 position PreON is available for use with TMTpro). For iST workflows, up to three runs per day to give a total of 36 samples are feasible. For iST-NHS workflows, up to two runs per day to give a total of 24 samples are feasible.

9.4. Can the PreON prepare both label-free and chemical labeling samples?

Yes, the PreON can execute workflows for both label-free and chemical labeling samples with our ready-to-go iST, iST-BCT and iST-NHS kits.

9.5. What tubes should be used in the PreON for samples?

We recommend the use of 1.5 mL lo-bind Eppendorf tubes (Catalog No. 0030108442). Tubes that are not the correct dimensions will cause pipetting errors.

9.6. Can I run my own methods on the PreON?

The PreON works seamlessly with our ready-to-go iST, iST-BCT and iST-NHS kits. Other methods or commercial products cannot be used with the PreON.

9.7. Which kind of maintenance does the PreON require?

The PreON requires minimal maintenance such as cleaning the surfaces and emptying the solid and liquid waste containers. Once every six months we recommend performing a tightness test for the pipetting unit (all required tools provided).

9.8. How is the PreON serviced?

Our global and trusted partner PEAK-Service is the leading global provider for technical services for medical, analytical and industrial equipment. Contact us at info@preomics to inquire about further servicing options and pricing.

10. KIT SHIPPING AND STORAGE

10.1. Does PreOmics ship worldwide?

Yes, we do ship our products worldwide. For the convenience of our US customers, we ship from our warehouse in New Jersey.

10.2. How do you ship your products and how shall I store them?

We ship at ambient temperature. Upon arrival, please store the lyophilized enzyme mix (red DIGEST tubes) at -20°C and the rest of the kit at room temperature.

10.3. Can I freeze whole kits upon arrival?

No, freezing is detrimental to our buffers. Only the lyophilized enzymes (red DIGEST tubes) in the iST, iST-REG-PSI, iST-NHS and iST-BCT kits should be frozen upon arrival for long-term storage.

10.4. How do I store resuspended DIGEST in case I have leftover solution?

Resuspended DIGEST can be stored at 4°C for up to two weeks. For long-term storage, lyophilize the DIGEST again, lyophilized DIGEST can be stored at -20°C for up to nine months.

10.5. How long can I store the PreOmics' kits?

We guarantee a minimum remaining shelf life of three months after receiving our products. Please refer to the shelf life information printed on each kit box for further details.

10.6. Can I still use a kit after its shelf life has expired?

The performance of our LYSE / LYSE-NHS / LYSE-BCT will drop significantly after the expiration date. Thus, we do not recommend to use our kits after the shelf life has expired.

11. ACCESSORIES

11.1. What is the Metal Heating Shaker Adapter?

The Metal Heating Shaker Adapter guarantees optimal heat transfer for our CARTRIDGES compared to planar heating systems. It is compatible with any heating shaker in the SPSS format and many liquid handling platforms. It is also directly compatible with our 96-well CARTRIDGE adapter plates.

11.2. When purchasing the PreOmics' 96 reaction kits, do I need to order the 96-well adapter plate too?

All our kits in the 96 reaction format already contain the 96-well adapter plate required for convenient handling of larger sample numbers.

12. ORDERING

12.1. How can I order your products?

Customers from North America please order via PreOmics Inc. (USA), customers from the rest of the world please order via PreOmics GmbH (Germany).

We offer several options to order our products:

- Send an email to: order@preomics.com
- Call us +49-89-2314-163-0
- Fax us +49-89-2314-163-99

12.2. Is it possible to order individual items from your kits?

We provide complete solutions to ensure best results for your LC-MS/MS analyses. Adaptions to our lysis buffers might be necessary though for specific experimental questions. Upon request, we provide the appropriate 2x concentrated LYSE buffer and the WASH0 for use with plant, algae and diatom samples.

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