Frequently asked questions (FAQs)
iST Sample Preparation Technology

PREOMICS

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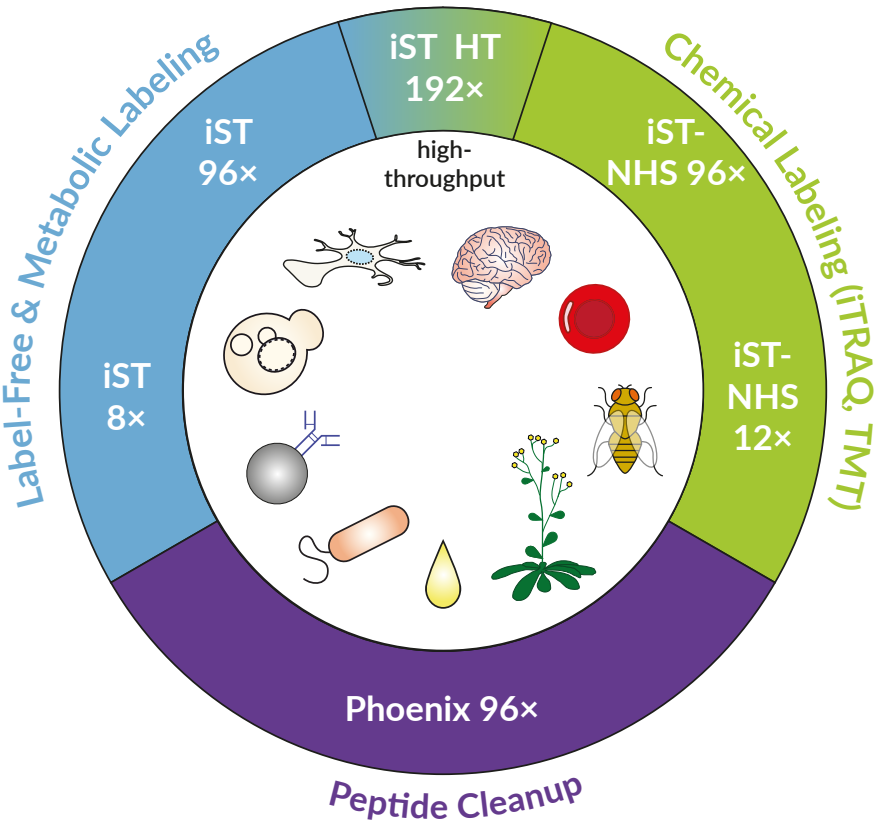
INTRODUCTION

Complex sample preparation workflows, extensive fractionation and proteolytic digestion are highly time consuming and restrict the overall technical reproducibility. PreOmics addresses these limitations and our iST technologies enable a robust and reproducible sample preparation with a significant time advantage compared to other commonly employed methods, which is essential for reliable and informative results.

The iST kits are compatible with label-free and metabolic labeling (e.g. SILAC). The iST-NHS kits are compatible with chemical labeling (e.g. iTRAQ or TMT). Both, the iST and iST-NHS kits are complete sample preparation kits to process whole cells, tissues, body fluids, precipitated proteins and other sample types.
The PHOENIX kit is a peptide cleanup kit and the input material is a peptide sample. The PHOENIX offers an easy-to-use solution to clean up peptide mixtures and efficiently remove detergents, polymers, salts, lipids and more for reliable LC-MS analyses.

The Versatility of iST



FREQUENTLY ASKED QUESTIONS

1. SAMPLE PREPARATION

1.1 Is my own lysis buffer compatible with the iST / iST-NHS kits?

We strongly recommend to use the LYSE or LYSE-NHS buffers provided with the iST or iST-NHS kits, respectively. If you want to use your own lysis buffer, the efficiency of cell lysis and thus protein identification are likely to suffer. Please refer to the following table or contact us (info@preomics.com) to discuss the compatibility of your lysis buffer with the iST technology:

own lysis buffer	iST / iST-NHS compatible?	max. vol. own lysis buffer	remarks
PBS	yes	25 µL	dilute with 25 µL 2X concentrated LYSE / LYSE-NHS
RIPA (max. 0.1% SDS, 1% SDC, 1% Triton x-100)	yes	25 µL	dilute with 25 µL 2X concentrated LYSE / LYSE-NHS
urea (max. 2M; no thiourea)	yes	25 µL	dilute with 25 µL 2X concentrated LYSE / LYSE-NHS
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 my sample to be processed with the iST / iST-NHS kits?

Your sample volume (liquid or solid, e.g. cell pellets) should not exceed 10 µL when combined with our LYSE / LYSE-NHS buffers. For sample volumes between 10-25 µL, we recommend use of our 2-fold concentrated lysis buffers (2X LYSE, 2X LYSE-NHS), which can be purchased upon request. If your sample volume exceeds 25 µL, we recommend to perform protein precipitation. Precipitated proteins can subsequently be processed with our regular iST or iST-NHS workflows.

1.3 How do I perform protein precipitation?

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

1. Transfer protein lysate (not more than 300 µL) to a clean 2 mL Eppendorf tube.
   > in case you have a larger sample volume, use either a 5 or 15 mL tube
2. 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
3. Mix briefly
4. Incubate for one hour at -20°C
5. Spin in table-top centrifuge at 4°C for 15 min at 13,000 rpm
6. Carefully discard supernatant, make sure not to disturb the pellet
7. Air-dry the pellet for 5-10 min
8. Continue with PreOmics iST protocol: add 50 µL LYSE buffer, follow the iST protocol from here on
   > you can also freeze the pellet after air-drying at -20°C or -80°C until further use

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 disruption in combination with the iST / iST-NHS kits?
For samples such as cells, yeast or tissue material, we recommend to perform mechanical force disruption in the presence of our LYSE or LYSE-NHS buffers. Many different mechanical force methods can be employed such as traditional bead milling, liquid nitrogen grinding, or commerical systems from various vendors (e.g. Bertin Instruments, Covaris, Hielscher, MP Biomedicals). We recommend the Bioruptor® Pico system (Diagenode), that allows direct placement of our CARTRIDGES.
Samples such as body fluids do not require additional mechnical force disruption.

1.6 What are the minimum and maximum protein amounts recommended for the iST / iST-NHS kits?
We get highly reproducible results with protein starting amounts ranging from 1 µg to 100 µg. For low input information please see 1.10.

1.7 What is the maximum volume I can load on the PreOmics CARTRIDGES?
The maximum volume is 250 µL.

1.8 How much raw material (cells, tissues, body fluids, ...) do I need for the iST / iST-NHS kits?
Protein content varies considerably across distinct biological input material (e.g. different cell lines, strains, tissues, tissue regions, body fluids) and sample storage conditions (e.g. different storage temperatures, storage time, repeated freeze-thaw cycles, fresh frozen vs. FFPE, formaldehyde treatment). Hence, we always recommend to quantify protein concentration of your sample after the lysis (see 1.9). As a rule of thumb, a short overview of recommended raw material amounts is given in the table below.

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 / CSF (*H.sapiens*)	2 µL	100 µg
Urine (*H.sapiens*)	100 mL*	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

*requires concentration, please refer to our urine-specific protocol

1.9 Which protein quantification methods are compatible with the iST / iST-NHS Kits?
Most classical assays are compatible with our LYSE and LYSE-NHS buffers. We recommend the BCA assay or the tryptophan quantification method. Some assays require dilution with distilled water to achieve best results:

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

1.10 Shall I adjust the buffer volumes depending on the protein starting amounts?
For 20 µg protein starting material or less, add 10 µL LYSE and 10 µL DIGEST to your sample, keep all other buffers as indicated in the protocol. Accordingly, you may adjust the volumes for chemical labeling and quenching as recommended by the label manufacturer.

1.11 Which lysis temperature shall I use?
We recommend to perform the lysis/denaturation at 95°C and to use lower temperatures only for temperature-sensitive samples such as immunoprecipitations. We have tested lower temperatures down to 60°C and do not see any differences in parameters assessed (e.g. IDs, alkylation rates).

1.12 How long shall I digest my samples?
The digestion depends on your sample type and input material (see table below). Please note our recommendations to lower the enzyme amount for low input samples (<20 µg protein starting material, see 1.10).

Sample Type	Digestion Time
Precipitated proteins	1-3 hrs
Cell lines	1-3 hrs
Body 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 Can I use other enzymes for the protein digestion?
Currently, our iST and iST-NHS kits come with a lyophilized enzyme mix consisting of LysC and trypsin. We plan to develop solutions for other enzymes as well.

1.14 Are the iST / iST-NHS workflows compatible with enrichment of phosphorylation sites?
While our protocols are in general compatible with IP samples (modified protocols upon request or to be found for download on our website), the required input amounts for global phosphorylation enrichment experiments (~500 µg) usually exceed the peptide binding capacity of our CARTRIDGES. We plan to develop CARTRIDGES with higher binding capacities for applications with post-translational modifications.

1.15 How can I automate my sample processing efforts?
We are collaborating with many different providers of liquid handling systems to offer the best automation solutions for your needs. For platform-specific protocols and more information on either semi- or full-automation please contact us at info@preomics.com.

1.16 Are the PreOmics kits compatible with absolute quantification?
All of our kits (iST, iST-NHS, Phoenix) are compatible with absolute quantification strategies. For absolute quantification employing isotopically-labeled protein standards with our iST and iST-NHS kits, please add the respective absolute standard (e.g. DIGESTIF, PSAQ, SILAC-PrEST) together with your sample to the LYSE or LYSE-NHS buffer and proceed with the protocol accordingly.
For absolute quantification employing isotopically-labeled peptide standards with either the iST, iST-NHS or Phoenix kits, please introduce the respective standard (e.g. AQUA, QconCAT) before adding the STOP buffer to your samples.

1.17 How do I assess the peptide recovery rate after the whole sample processing workflow?
The best way to assess peptide recovery rates is by employing absolute quantification strategies (see 1.15). Our peptide recovery is >80% over a range of 1-100 µg protein input.

2. CHEMICAL LABELING (iTRAQ, TMT)

2.1 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 ($C_6H_{11}NO$), specificity [C], mass shift +113.084 Da

Please note that samples prepared with the iST and the iST-NHS kit cannot be processed together in the very same data analysis workflow, since they contain two mutually exclusive, fixed modifications on cysteine.

2.2 How can I improve the labeling efficiency when performing chemical labeling in combination with the iST-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 recommendations:

> 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.

> We recommend to only use fresh labeling reagents. 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.

2.3 Shall I perform the chemical labeling step on the cartridge or in the tube?

We recommend to perform the labeling in an Eppendorf tube and only to transfer the labeled peptides to our CARTRIDGES for the final peptide cleanup.

2.4 When shall I mix channels after the chemical labeling?

We recommend to measure the peptide concentration after the peptide cleanup step and then to mix the channels accordingly.

3. PEPTIDE CLEANUP

3.1 Which sample types require extra washing buffers?

Most peptide samples can be cleaned up with the washing buffers presented in the iST / iST-NHS 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. For cleaning up peptide samples with stronger sources of contamination, e.g. sugars, fat, polymers or high concentrations of detergents, we recommend to use our PHOENIX peptide cleanup kit instead that features yet another washing buffer called „WASHX“.

3.2 What are the differences between the PreOmics washing buffers?

Washing Buffer	Organic	Acidic	Basic	Volatile	for which samples?
WASH0	yes	yes		yes	urine, plants
WASHX	yes	yes		yes	lipids, polymers, detergents
WASH1	yes	yes		yes	hydrophobic contaminations
WASH2		yes		yes	hydrophilic contaminations

3.3 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 MTP plate, or you can stack it on top of standardized autosampler vials.

3.4 How long do I need to concentrate my samples in the SpeedVac?

We recommend to 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 SpeedVac.

3.5 Can I concentrate iST / iST-NHS eluates together with samples eluted from C18 columns in the very same SpeedVac?

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

3.6 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 the NanoDrop instrument to save your precious peptide samples.

3.7 How can I resuspend dried peptides in the 96-well plate?

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

3.8 How shall 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.

4. PHOENIX KITS

4.1 Are the CARTRIDGES from the iST / iST-NHS and the PHOENIX kits the same?
CARTRIDGES in the iST and iST-NHS kits are the same. CARTRIDGES in the PHOENIX kit are of different nature and have a slightly lower affinity for hydrophobic species.

4.2 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. To do so, 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.

5. KIT SHIPPING & STORAGE

5.1. Does PreOmics ship worldwide?
Yes, we do ship our products worldwide. For the convenience of our US customers, we drop-ship US deliveries from our warehouse in New Jersey. Customers in the following countries please refer to our distributors listed at the very end of this document: Australia, Japan.

5.2 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.

5.3 Can I freeze whole kits upon arrival?
No, freezing is detrimental to our buffers. Only the lyophilized enzymes (red DIGEST tubes) in the iST and iST-NHS kits should be frozen upon arrival for long-term storage.

5.4 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, we recommend to lyophilize the DIGEST again. Lyophilized DIGEST can be stored at -20°C for up to nine months.

5.5 How long can I store the iST / iST-NHS kits and PHOENIX kits?
We guarantee a shelf life of at least nine months after production. Please refer to the shelf life information printed on each kit box for further details.

6. ACCESSORIES

6.1 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.

6.2 When purchasing the iST / iST-NHS or PHOENIX 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.

7. ORDERING

7.1 How can I order your products?

We offer several options to order our products:

- Send an email to: order@preomics.com
- Call us at +49-89-2314-163-0
- Fax us at +49-89-2314-163-99
- Visit our webshop at www.preomics.com/products

7.2 How does the PreOmics webshop work?

1. Go to www.preomics.com/products
2. Select your products of choice and add them to your shopping cart
3. Select your delivery and billing address
4. Shipping costs and taxes will be added during the checkout procedure
5. Pay by credit card or PayPal

7.3 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 following buffers: LYSE, 2X LYSE (2-fold concentrated), LYSE-NHS, 2X LYSE-NHS (2-fold concentrated).

8. PAYMENT

8.1 Which payment options do you offer?

We prefer wire transfers to our German bank account:

Name: PreOmics GmbH
IBAN: DE29 7004 0048 0794 1297 00
Bank: Commerzbank MUE GF-M48
BIC: COBADEFFXXX

We also accept electronic funds transfer (ACH) to our US distribution company`s (BIOjump) account:

TD Bank N.A.
Reference: Preomics ("Invoice #")
Account No: 4317788292
ABA Routing: 031201360
SWIFT: NRTHUS33XXX

Or by cheque to:

BIOjump LLC
1 Jill Court, Building 16 Unit 10
Hillsborough, NJ 08844
USA

Customers using our webshop can also pay directly by credit card or PayPal (see 7.2).

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