Sample Preparation Kit
For reproducible mass spectrometry proteomics

MANUAL

© Biognosys AG, Switzerland
Table of Contents

Sample Preparation Kit Components ................................................................. 3
Storage and Quality Control of Sample Preparation Kit .................................... 3
Use Limitations ..................................................................................................... 3
Product Warranty and Satisfaction Guarantee .................................................... 4
Technical Assistance ............................................................................................ 4
Safety Information ................................................................................................. 4
Introduction: Sample Preparation Kit .................................................................. 5
Important Notes before Start ................................................................................. 5
Additionally Required Laboratory Equipment and Consumables ...................... 6
Additionally Required Reagents, Solvents and Solutions .................................... 6
Plasma Sample Preparation Procedure ................................................................. 7
Cell Culture Sample Preparation Procedure ....................................................... 9
Trademarks & Limited License Agreement ........................................................... 10
Sample Preparation Kit Components

<table>
<thead>
<tr>
<th>Sample Preparation Kit</th>
<th>Part No: Ki-3010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sufficient for</td>
</tr>
<tr>
<td></td>
<td>preparation of 50</td>
</tr>
<tr>
<td></td>
<td>samples</td>
</tr>
<tr>
<td>Alkylation Solution</td>
<td>1x 10 ml tube, yellow</td>
</tr>
<tr>
<td>Reduction Stock Solution</td>
<td>1x 0.5 ml tube, orange</td>
</tr>
<tr>
<td>LC Solution</td>
<td>1x 10 ml tube, clear</td>
</tr>
<tr>
<td>10x Dilution Buffer</td>
<td>1x 10 ml tube, green</td>
</tr>
<tr>
<td>Denature Buffer</td>
<td>1x 10 ml tube, violet</td>
</tr>
</tbody>
</table>

Storage and Quality Control of Sample Preparation Kit

After receiving the kit store all components at +4°C and **protected from light**.

In accordance with Biognosys’ Quality Management System each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

Use Limitations

Sample Preparation Kit is intended only for research use in mass spectrometry proteomics applications. This product is not intended for the diagnostic, prevention, or treatment of a disease. All due care and attention should be exercised in the handling of the products.
Product Warranty and Satisfaction Guarantee

Biognosys guarantees the performance of the product when following the instructions and protocols described in this product manual. However, the user must determine the suitability of the product for its particular use. Should the product fail to perform satisfactorily due to any reason other than misuse, Biognosys will replace it free of charge. Biognosys reserves the right to change, alter, or modify any product or component thereof to enhance its performance and design.

If you have questions about product specifications or performance, please contact us at support@biognosys.com. We also encourage you to contact us if you have any suggestions for improving product performance or for its use in new applications and techniques.

Technical Assistance

Our technical department is composed of experienced scientists with extensive practical and theoretical expertise in proteomic technologies. If you have any questions or experience any difficulties with the Sample Preparation Kit please do not hesitate to contact us at support@biognosys.com, call +41 44 738 20 40 or visit www.biognosys.com/shop/sample-preparation-kit.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the material safety data sheet (MSDS) available online in convenient and compact PDF format at www.biognosys.com/shop/sample-preparation-kit.

The following risk and safety phrases apply to components of Sample Preparation Kit.

10X Dilution Buffer: Harmful if swallowed.

Alkylation Solution: Toxic if swallowed, may cause allergy or asthma symptoms or breathing difficulties if inhaled, may cause an allergic skin reaction, may cause long lasting harmful effects to aquatic life.

Reduction Stock Solution: Harmful if swallowed, causes skin and eye irritation, may cause respiratory irritation.
Introduction: Sample Preparation Kit

Mass spectrometry based proteomics has become a prominent tool in biology and medicine in recent years due to its ability to identify and to precisely quantify thousands of proteins from different samples.

However, variations in sample preparation between experiments and individual samples remain an enormous source of experimental bias, especially in high-throughput applications. Since the proteomes of natural samples are extremely complex with typically 100'000s of peptides present, spanning over several orders of magnitude, it is challenging to establish a reliable method for preparing protein samples for analysis by mass spectrometry.

Biognosys’ Sample Preparation Kit is a simple-to-use standardized kit for reproducible mass spectrometry proteomics of mammalian cell cultures and plasma/serum samples. The protocols work well with all mass spectrometry based proteomics applications such as SRM/MRM/PRM, HRM/DIA/SWATH and shotgun MS/MS.

Important Notes before Start

Before starting with the sample preparation read through the steps carefully and make sure all the required reagents and equipment are available.

Use LC-grade solvents and water throughout the protocol to prepare buffers and solutions.

The kit components are sufficient for 50 plasma or 50 cell culture sample preparations to be made at once. Buffers are unstable and thus not appropriate for long-term storage once they are dissolved.

Sample preparation using this kit lasts a couple of hours, however, the protocol includes an overnight incubation!
### Additionally Required Laboratory Equipment and Consumables

<table>
<thead>
<tr>
<th>Equipment/Consumables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single channel pipettes (0.5 µl – 1000 µl) with corresponding tips</td>
</tr>
<tr>
<td>Glass syringe (100 µl)</td>
</tr>
<tr>
<td>pH paper (universal pH indicator paper pH 1-10 with colour scale) or pH meter with small combined glass electrode</td>
</tr>
<tr>
<td>Vortex mixer</td>
</tr>
<tr>
<td>15 ml plastic tubes</td>
</tr>
<tr>
<td>Benchtop centrifuge</td>
</tr>
<tr>
<td>Thermomixer at +37°C</td>
</tr>
<tr>
<td>Vacuum centrifuge</td>
</tr>
<tr>
<td>LC-MS vials</td>
</tr>
</tbody>
</table>

### Additionally Required Reagents, Solvents and Solutions

<table>
<thead>
<tr>
<th>Reagents/Solvents/Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequencing grade modified trypsin, stock solution at 0.4 µg/µl (recommended Promega Product Catalog#V5113)</td>
</tr>
<tr>
<td>10% (v/v) TFA solution, prepare at least 2 ml (Note 1, Note 2)</td>
</tr>
<tr>
<td><strong>Strongly Recommended:</strong> C18 clean-up assay (commercially available from e.g. The Nest Group Inc)</td>
</tr>
<tr>
<td>Methanol, at least 20 ml</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Acetonitrile</td>
</tr>
</tbody>
</table>

**Note 1**
Solution can be stored at room temperature for up to one year.

**Note 2**
Use a glass syringe to pipette strong acidic solutions like concentrated TFA.
Plasma Sample Preparation Procedure

Sample Requirements
Plasma or serum, 10 µl per sample.

A. Denaturation, reduction and alkylation

1. Dissolve 10x Dilution Buffer with water to a total volume of 5 ml (Note 3), vortex until solubilized.
2. Dissolve Reduction Stock Solution with 250 µl water, vortex, briefly spin down.
3. Prepare Denature Buffer:
   3.1. Add 500 µl of 10x Dilution Buffer to the Denature Buffer tube. Keep 10x Dilution Buffer in fridge until further usage in step 14.
   3.2. Add 25 µl of Reduction Stock Solution to the Denature Buffer tube.
   3.3. Fill up Denature Buffer with 2.3 ml of water (Note 3, Note 4).
4. Add 80 µl/sample of Denature Buffer to a new 1.5 ml tube.
5. Add 10 µl of a plasma sample to each tube (Note 5).
6. Gently shake the samples on the thermomixer for 1 min at room temperature.
7. Briefly spin down to collect all liquid at the bottom of the wells.
8. Incubate the samples at +37°C, 600 rpm on the thermomixer for 30 min.
9. Let the samples cool to room temperature while preparing the Alkylation Solution:
   9.1. Fill up Alkylation Solution with water to 3 ml (Note 3), vortex, briefly spin down (Note 6).
10. Add 16 µl/sample of Alkylation Solution.
11. Gently shake the samples on the thermomixer for 1 min at room temperature.
12. Briefly spin down to collect all liquid at the bottom of the wells.
13. Incubate the samples at room temperature in the dark for 30 min.

B. Dilution

14. Prepare 1x Dilution Buffer by mixing in a separate tube 1 ml of 10x Dilution Buffer and 9 ml of water.
15. Add 50 µl/sample of 1x Dilution Buffer to a new 1.5 ml tube.
16. Add 3 µl/tube of denatured plasma sample (from Section A, step 13).
   Optional: you might freeze the remaining of samples for a repeated analysis.
17. Check pH to be 8–9 in a few samples by pipetting 0.5 µl onto a...
pH paper or by using a pH-electrode (Note 7).

17.1. If pH is below 8, adjust it in all samples using 5 µl of 10x Dilution Buffer.

17.2. Check again in a few wells, repeat steps 17.1 and 17.2 if necessary.

C. Digestion using endoprotease trypsin

18. Thaw trypsin (0.4 µg/µl) and spin down briefly.
19. Add 1 µl of trypsin to each sample from Section B.
20. Gently shake the samples and then briefly spin down to collect all liquid at the bottom of the tubes.
21. Incubate the samples for 3 hours at +37°C, 600 rpm in the thermomixer.
22. Acidify samples by adding 20 µl/sample of 10% (v/v) TFA solution (Note 8).
23. Check pH to be below 2 in a few samples by pipetting 0.5 µl onto a pH paper or by using a pH-electrode.

23.1. If pH is above 2, adjust it in all samples adding 5 µl of 10% (v/v) TFA solution.

23.2. Check again in a few samples, repeat steps 23.1 and 23.2 if necessary.
24. Gently shake the samples for 1 min at room temperature.
25. Centrifuge the samples at 10’000 x g for 10 min.
26. Carefully transfer the supernatant to a new 1.5 ml tube for further use and discard the precipitate. **IMPORTANT:** At this point we strongly recommend to perform a C18 clean-up procedure (commercially available from e.g. The Nest Group Inc) to prevent the clogging of your LC columns.
27. Dry-vacuum the samples, each sample contains approximately 20 µg of peptides.
28. Add 20 µl/sample of LC Solution, briefly vortex and transfer the re-suspend samples into LC-MS vials.
29. Samples are now ready for injection and can also be stored at -20°C if convenient.

**Note 7**
Avoid sample cross-contamination by using fresh tips or rinsing the electrode with water for every sample.

**Note 8**
Foaming is possible; add 10% (v/v) TFA solution slowly.
Mammalian Cell Culture Sample Preparation Procedure

Sample Requirements
Cell culture pellets, with at least 100 µg of total protein per sample (approximately 100'000 to 1'000'000 cells depending on the cell line). If possible do not use trypsin for collection of adherent cells as cell surface proteins are damaged (use cell scraper instead).

A. Denaturation
1. Dissolve 10x Dilution Buffer with water to a total volume of 5 ml (Note 9), vortex until solubilized.
2. Prepare Denature Buffer:
   2.1. Add 500 µl of 10x Dilution Buffer to the Denature Buffer tube. Keep 10x Dilution Buffer in fridge until further usage.
   2.2. Fill up Denature Buffer with 2.3 ml of water (Note 9, Note 10).
3. Add 80 µl of Denature Buffer to each sample and vortex until cell pellet is re-suspended (approximately 1 min).
4. Leave the samples on room temperature for 30 min.  
   Optional: Transfer one drop of sample on a glass slide and check lysis with a microscope. If intact cells are still visible, additional lysis measures, like bead milling or ultra-sonication might be necessary
5. During incubation you can measure the total protein concentration using a total protein assay (like BCA or Bradford) according to the manufacturer’s protocol.
6. Prepare 1x Dilution Buffer by mixing in a separate 15 ml tube 1 ml of 10x Dilution Buffer and 9 ml of water.
7. Dilute the samples with 1x Dilution Buffer to obtain 100 µl per sample with protein concentration of 1 µg/ µl. Keep 1x Dilution Buffer in fridge until further usage.  
   Optional: If convenient you can freeze your samples on -20°C. After thawing vortex the samples for 2 min before further use.

B. Reduction and alkylation
8. Dissolve Reduction Stock Solution with 250 µl water, vortex, briefly spin down.
9. Add 0.5 µl of Reduction Stock Solution to each sample.
10. Incubate the samples at +37°C, 600 rpm on the thermomixer for 30 min.
11. Let the samples cool to room temperature while preparing the Alkylation Solution:  
   11.1. Fill up Alkylation Solution with water to 3 ml (Note 9), vortex, briefly spin down (Note 11).
13. Briefly vortex and spin down to collect all liquid at the bottom of the vials.
14. Incubate the samples at room temperature in the dark for 30 min.

Note 9  
To solubilize the provided solid components, dissolve first in a small amount of the stated solvent and fill-up to final volume carefully.

Note 10  
Warm up the tube to help solubilizing the reagent by holding the tube in warm (<40°C) tap water.

Note 11  
Light sensitive, prepare shortly before usage and keep in dark.
15. Add 25 µl of each sample to a new tube and dilute it with 25 µl/sample of 1x Dilution Buffer.
   Optional: you might freeze the remaining of samples of denatured cell lysate for a repeated analysis.

16. Check pH to be 8–9 in a few samples by pipetting 0.5 µl onto a pH paper or by using a pH-electrode (Note 12).
   16.1. If pH is below 8, adjust it in all samples using 5 µl of 10x Dilution Buffer.
   16.2. Check again in a few tubes, repeat steps 16.1 and 16.2 if necessary.

C. Digestion using endoprotease trypsin

17. Thaw trypsin (0.4 µg/µl) and spin down briefly.
18. Add 1 µl of trypsin to each sample from Section B.
19. Incubate the samples overnight at +37°C, 600 rpm in the thermomixer.
   **NEXT DAY:**
20. Acidify samples by adding 20 µl/sample of 10% (v/v) TFA solution using a pipette (Note 13).
21. Check pH to be below 2 in a few samples by pipetting 0.5 µl onto a pH paper or by using a pH-electrode.
   21.1. If pH is above 2, adjust it in all samples adding 5 µl of 10% (v/v) TFA solution.
   21.2. Check again in a few samples, repeat steps 21.1 and 21.2 if necessary.
22. Centrifuge the samples at 10’000 x g for 10 min.
23. Carefully transfer the supernatant to a new 1.5 ml tube for further use and discard the precipitate.
   **IMPORTANT:** At this point we strongly recommend to perform a C18 clean-up procedure (commercially available from e.g. The Nest Group Inc) to prevent the clogging of your LC columns.
24. Dry-vacuum the samples, each sample contains approximately 20 µg of peptides.
25. Add 20 µl/sample of LC Solution, briefly vortex and transfer the re-suspend samples into LC-MS vials.
26. Samples are now ready for injection and can also be stored at -20°C if convenient.

**Note 12**
Avoid sample cross-contamination by using fresh tips or cleaning the electrode with water for every sample.

**Note 13**
Foaming is possible; add 10% (v/v) TFA solution slowly.

**Trademarks & Limited License Agreement**

Biognosys is a registered trademark in Switzerland and other countries. Information in this document is subject to change without notice. Biognosys assumes no responsibility for any errors that may appear in this document. In no event shall Biognosys be liable for incidental, special, multiple, or consequential damages in connection with or arising from the use of this document, Biognosys’ Sample Preparation Kit and any of its components. For general chemical safety information, background information, and detailed procedures, please refer to www.biognosys.com/shop/sample-preparation-kit.
Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of Sample Preparation Kit to the following terms:

1. Sample Preparation Kit may be used solely in accordance with the Sample Preparation Kit Manual and with components contained in the kit only. Biognosys grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the Sample Preparation Kit Manual.

2. Other than expressly stated licenses, Biognosys makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.

3. The Sample Preparation Kit and its components are licensed for 50 samples (one-time use) and may not be reused, refurbished, or resold.

4. Biognosys specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of this kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. Biognosys may enforce the prohibitions of this Limited License Agreement in any court, and shall recover all its investigative and court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

© 2017 Biognosys AG, all rights reserved.