Sample Preparation Kit

For reproducible mass spectrometry proteomics

MANUAL

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Sample Preparation Kit Components

Sample Preparation Kit	Part No: Ki-3010 Sufficient for preparation of 50 samples
Alkylation Solution	1x 10 ml tube, yellow cap
Reduction Stock Solution	1x 0.5 ml tube, orange cap
LC Solution	1x 10 ml tube, clear cap
10x Dilution Buffer	1x 10 ml tube, green cap
Denature Buffer	1x 10 ml tube, violet cap

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

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

Additionally Required Reagents, Solvents and Solutions

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

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

Note 3
To solubilize the provided solid components, dissolve first in a small amount of the stated solvent and fill-up to final volume carefully.

Note 4
Warm up the tube to help solubilizing the reagent by holding the tube in warm (<40°C) tap water.

Note 5
Thawed plasma samples should not be left without Denature Buffer at room temperature for longer than 5 minutes.

Note 6
Light sensitive, prepare shortly before usage and keep in the dark.

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.

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

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 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 -20ºC. After thawing vortex the samples for 2 min before further use.

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.

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).
12. Add 16 µl/sample of Alkylation Solution.
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 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.

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

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

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