

In Solution Digest for Mass Spectrometry (MS) Analysis

Matthias Mann

Max Planck Institute for Biochemistry, Department of Proteomics and Signaltransduction,
Martinsried, Germany

INTRODUCTION

There are two major strategies for converting proteins extracted from biological material to peptides suitable for MS-based proteome analysis. The first involves solubilization of proteins with detergents, separation of proteins by SDS-PAGE (**S**odium **D**odecil **S**ulfate **P**oly**a**crylamide **G**el **E**lectrophoresis), and digestion of the gel-trapped proteins ('in-gel' digestion). The second is the detergent-free method comprising protein extraction with strong chaotropic reagents such as urea and thiourea, protein precipitation, and digestion of proteins under denaturing conditions ('in-solution' digestion). For samples containing no detergent and of low to medium complexity (<300 proteins), in-solution digestion can be used and will provide similar results to in-gel digestion. This technique can also be very useful when working with small protein amounts, since the sample recovery is better than that for in-gel digestion.

MATERIALS

- DENATURATION BUFFER: 6 M urea/2 M thiourea in 10 mM HEPES (pH 8.0). To make 10 mL, dissolve 3.6 g urea, 1.52 g thiourea and 23.83 mg HEPES in ~4 mL water, mixing vigorously. Adjust pH with NaOH. Complete the volume to 10 mL with water. Centrifuge the solution at 5,000 x g for 10 min to remove insoluble particles. Store in small aliquots at -80°C.
- DIGESTION BUFFER: 50 mM ammonium bi-carbonate (NH₄HCO₃, ABC) in water (pH 8.0). To make 10 mL, dissolve 40 mg ABC in 10 mL water. Store at room temperature.
- REDUCTION BUFFER: 10 mM dithiothreitol (DTT) in 50 mM ABC. To make 10 mL, dilute 100 µL of a 1 M DTT solution in 9.9 mL digestion buffer. Stored in small aliquots at -20°C.
- ALKYLATION BUFFER: 55 mM iodoacetamide in 50 mM ABC. To make 10 mL, dissolve 102 mg iodoacetamide in 10 mL digestion buffer. Store in small aliquots at -20°C. *Keep in the dark!*
- LYSC SOLUTION: 0.5 µg/µL LysC in 50 mM ABC. To prepare 10 mL, dissolve 5 mg LysC in 10 mL digestion buffer. Store in small aliquots at -20°C.
- TRYPsin SOLUTION: 0.5 µg/µL trypsin in 50 mM ABC. When diluting one new vial containing 20 µg trypsin (modified sequencing grade, Promega), measure the original volume and add enough digestion buffer to reach 40 µL final volume. Prepare IMMEDIATELY before use and keep it always on ice to minimize autocatalysis. Stored in small aliquots at -20°C.
- 100% TFA

METHODS

General remark:

In this procedure all steps are done at room temperature to reduce unwanted derivatization of amino acid side-chains by the denaturing agents. So, NEVER heat your sample!

1. Solubilize the proteins in the denaturation buffer. Keep the final volume as small as possible. When proteins are contained in large volumes, the denaturants can be added directly to the sample, to avoid dilution of the denaturation buffer. Use enough urea/thiourea to have the desired concentration in 2-fold the original sample volume, since these compounds increase in volume when dissolved. The pH of the final solution should be near 8.0. Adjust if necessary.

2. Add 1 μL DTT / 10 μL digestion solution and incubate for 30 min at room temperature;
3. Add 1 μL iodoacetamide / 10 μL digestion solution and incubate for 20 min at room temperature;
4. Add 1 μg LysC solution / 50 μg protein and incubate for at least 3 h at room temperature;
5. Dilute sample with $\sim 4\times$ with digestion buffer;
6. Add 1 μg trypsin/50 μg sample protein and incubate overnight at room temperature.
7. Stop the digestion by acidifying the sample to $\text{pH} < 2.5$ with 100% TFA.
8. Proceed the peptides purification using StageTips (see protocol). Digested peptides can also be stored at -20°C until use.

BIBLIOGRAPHY

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