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► **To cite this version:**

Leslie Muller, Luc Fornecker, Sarah Cianférani, Christine Carapito. Tube-Gel: A Fast and Effective Sample Preparation Method for High-Throughput Quantitative Proteomics. *Proteomics for biomarker discovery*, pp.123-127, 2019, 10.1007/978-1-4939-9164-8_8 . hal-02341533

HAL Id: hal-02341533

<https://hal.science/hal-02341533>

Submitted on 9 Oct 2020

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Tube-gel, a fast and effective sample preparation method for high-throughput quantitative proteomics

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Tube-gel sample preparation for high-throughput proteomics

Summary/abstract

Sample preparation is a key step in proteomics workflows. Tube-gel (TG) is a fast and repeatable sample preparation method that consists in the instantaneous trapping of the sample in a polyacrylamide gel matrix. It takes advantage of in-gel sample preparations by allowing the use of high concentrations of sodium-dodecyl sulfate but avoids the time-consuming step of electrophoresis. Therefore, TG limits the sample handling and is thus particularly suitable for high-throughput quantitative proteomics when large sample numbers have to be processed, as it is often the case in biomarker research and clinical proteomics projects.

Keywords

Tube-gel, sample preparation, high-throughput, detergent-compatibility, quantitative proteomics

1. Introduction

Tube-gel (TG) principle relies on the polymerization of polyacrylamide gel directly in the sample in a Laemmli-like solution. It was first introduced in 2005 by Lu X. *et al.* and consequently applied and adapted by others, in particular for the analysis of membrane proteins, lipid rafts proteins and the charge derivatization of peptides [1-8]. We have recently demonstrated its compatibility and repeatability for label-free quantitative proteomics by comparing it with a stacking gel and a standard urea-based liquid digestion protocol using

well-calibrated samples [9]. TG appeared to be comparable to classical in-gel sample procedure and over-performed liquid digestion. Major advantages of TG are the considerable gain of time and the limited sample handling it allows, thus reducing the introduction of biases and the risk of sample loss. Our method described in this chapter can be easily applied to the quantitative analysis of large sample cohorts during biomarker discovery and verification phases.

2. Materials

All solutions are prepared with ultrapure water.

2.1. Protein extraction

1. Tris-HCl 0.5 M, pH 6.8: weight 6 g of Tris in a glass bottle. Add 100 mL of water. Mix and adjust the pH with HCl. Store at 4 °C.
2. SDS: 10 % solution in water. Store at room temperature.
3. Glycerol: store at room temperature
4. Laemmli-like buffer: 62.5 mM Tris-HCl pH 6.8, 10 % glycerol (w/w), 2 % SDS (*see Note 1*). Leave one aliquot at 4 °C for current use and store the remaining aliquots at -20 °C (*see Note 2*).

2.2. Tube-gel preparation

1. Ammonium persulfate (APS): weight 20 mg of ammonium persulfate and add 200 µl of water (*see Note 3*)
2. N, N, N, N'-Tetramethylethylenediamine (TEMED): Store at room temperature.
3. 30 % acryl/bis-acrylamide: Store at 4 °C.
4. Eppendorf tube of 0.5 mL

5. Material for cutting the tube-gel: scalpel and a punch cutting 2 mm sections.
6. Fixation solution: 45 % (v/v) methanol, 5 % acetic acid (*see Note 4*). Store at room temperature.

2.3. Pre-digestion procedure

1. Ammonium bicarbonate 25 mM (NH_4HCO_3): weight 99 mg of ammonium bicarbonate and transfer to a plastic container. Add 50 mL of water and mix (*see Note 3*).
2. Dithiothreitol 10 mM (DTT): weight 15.4 mg of dithiothreitol and transfer to a plastic container. Add 10 mL of NH_4HCO_3 and mix (*see Note 5*).
3. Iodoacetamide 55 mM (IAA): weight 102 mg of iodoacetamide and transfer to a plastic container. Add 10 mL of NH_4HCO_3 and mix (*see Note 5*).
4. LC-grade acetonitrile (ACN)
5. Eppendorf tube of 2 mL

3. Methods

Carry out all procedures at room temperature unless otherwise specified.

3.1. Protein extraction

For simple procedure, extract proteins directly in the Laemmli-like buffer (*see Note 6*).

Extract recommended amounts of proteins between 10 and 100 μg in 30 μL of Laemmli-like buffer (final concentration 0.3 to 3 $\mu\text{g}/\mu\text{L}$).

3.2. Tube-gel preparation

1. Mix the 30 μL of extracted proteins in Laemmli-like buffer with 42.25 μL of ultrapure water, 25 μL of 30 % acryl/bis-acrylamide and 0.25 μL of TEMED in a 0.5 mL Eppendorf tube. Gently vortex and centrifuge the mixture until all bubbles have

disappeared (approximately 2,000 x g during 10 seconds). Add 2.5 μL of 10 % APS without introducing air bubbles and immediately briefly vortex and centrifuge the tube to remove residual bubbles (*see Note 7*).

2. After 1 hour, the TG has to be fixed with 200 μL fixation solution for at least 1 hour.
3. The TG is cut in 2 mm high sections and each section in $\sim 2 \text{ mm}^2$ pieces. Transfer the gel pieces into a 2 mL Eppendorf tube.

3.3.Pre-digestion procedure

1. Add 400 μL of 75 % ACN and 25 % NH_4HCO_3 at 25 mM to the gel pieces in order to wash them. Briefly agitate the tube and wait approximately 2 minutes before removing this solution. Repeat this step four times.
2. Dehydrate the gel pieces by adding 400 μL of ACN. Wait until the gel pieces are fully dehydrated (white color) and remove ACN.
3. Add 200 μL of 10 mM DTT in order to reduce the cysteine residues. Briefly agitate the tube and heat at 60 $^\circ\text{C}$ for 30 minutes. Then place the tube at room temperature for 30 minutes.
4. Add 200 μL of 55 mM IAA and place the tube in the dark for 20 minutes.
5. Add 400 μL of ACN. Briefly agitate the tube and remove the mixture contained in the tube.
6. In order to wash the gel pieces, add 200 μL of NH_4HCO_3 . Briefly agitate the tube and wait 5 minutes before adding 200 μL of ACN. Agitate the tube and remove the mixture. Repeat this step 3 times.
7. Finally dehydrate the gel pieces with 200 μL ACN. Wait until the gel pieces are fully dehydrated (white color) and remove ACN. Repeat this step twice (*see Note 8*).

4. Notes

Note 1. To prepare this solution, mix 0.5 mL of Tris-HCl 0.5 M, pH 6.8 with 0.8 mL of 10 % SDS solution, 0.4 mL of glycerol (It is also possible to weight glycerol because it is very viscous and difficult to pipette. In this case, weight 0.5 g of glycerol) and 2 mL of water.

Note 2. Bring the solution at room temperature before use as SDS precipitates at 4 °C.

Note 3. Prepare this fresh each time.

Note 4. To prepare this solution mix 450 mL of methanol with 50 mL of acetic acid and 500 mL of water in a glass bottle.

Note 5. Prepare this solution fresh. Wear a mask when weighing dithiothreitol and iodoacetamide. Handle this solution under the laboratory fume hood.

Note 6. Depending on the sample nature, heating, vortexing and/or sonication steps can be added.

Note 7. Caution should be taken with bubbles as air interferes with the polymerization process. All bubbles must be removed for an optimal polyacrylamide polymerization in order to avoid sample loss. Be careful, in these conditions the polymerization is fast and takes place in a few seconds.

Note 8. At this step, the sample can be store at – 20 °C prior to digestion. In this case, caution should be taken in order to remove all the liquid before freezing.

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