University of Teramo

Degree Course in Biotechnology - **Biochemical Methods – 2nd Year**

PRACTICAL SESSION 2 – Homogenization of a cell sample

Date: 2 November 2026

In this practical session you will extract total proteins from a cell pellet sample. The first step is to prepare the lysis buffer, a solution that disrupts cell membranes and solubilizes proteins. You will then follow the extraction protocol to obtain a clarified cell lysate, ready to be analysed.

Theoretical Background

Before you begin, answer the following questions.

Question: *What is meant by "cell lysis"? What kinds of forces hold the cell membrane together, and how does a detergent such as Triton X-100 manage to disrupt it?*

Answer: _____

_____

Question: *Why is a Protease Inhibitor Cocktail (PI) added to the lysis buffer? What would happen to the proteins without it?*

Answer: _____

_____

Question: *What does centrifugation at 14,000 g separate? What is found in the pellet and what in the supernatant after this centrifugation?*

Answer: _____

_____

Materials and Equipment

Reagents for the lysis buffer (final volume 50 mL):

- Tris-HCl pH 8 – 1 M stock solution
- NaCl – MW = 58.44 g/mol
- EDTA – 0.5 M stock solution
- $MgCl_2$ – 1 M stock solution
- Glycerol
- Triton X-100
- Distilled water

Reagents for the extraction:

- Lysis buffer
- Protease Inhibitor Cocktail
- Cell pellet

Equipment:

- Analytical and top-loading balance
- Graduated cylinders
- Beakers
- Micropipettes
- Pipette tips
- Eppendorf tubes
- Pestle
- Centrifuge
- Spatula
- Weighing boat

1. Preparation of the Lysis Buffer (50 mL)

The lysis buffer is a **physiological, buffered solution** that, thanks to the presence of the detergent Triton X-100, disrupts cell membranes and solubilizes proteins. The other components stabilize the pH, protect the proteins from degradation and maintain the correct ionic conditions.

> 📌 **Why these components?**
>
> **Tris-HCl pH 8:** buffers the solution at a slightly basic pH, optimal for protein solubility.
>
> **NaCl 150 mM:** provides physiological ionic strength, preventing non-specific interactions between proteins.
>
> **EDTA 1 mM:** chelates divalent ions (Ca^{2+}, Mg^{2+}), inhibiting metalloproteases and Mg^{2+}-dependent DNases.
>
> **$MgCl_2$ 1 mM:** cofactor required for certain enzymatic activities that are to be preserved.
>
> **Glycerol 10%:** stabilizes the proteins, protecting them from denaturation during the extraction process.
>
> **Triton X-100 1%:** non-ionic detergent that solubilizes cell membranes, releasing intracellular proteins.

Formulas for preparing the buffer:

For components from **a stock solution**, use: _____

For components from **a solid**, use: _____

Calculations and volumes/masses for the lysis buffer (50 mL)

Component	Final C	Stock / MW	Calculated value
Tris-HCl pH 8	50 mM	1 M	_____ mL
NaCl	150 mM	MW 58.44 g/mol	_____ g
EDTA	1 mM	160 mM	_____ µL
$MgCl_2$	1 mM	MW 95.21 g/mol	_____ g
Glycerol	10% v/v	100% (pure)	_____ mL
Triton X-100	1% v/v	10% stock	_____ mL
Distilled water	—	—	q.s. to 50 mL

Procedure - *Add the components in the order given; add the Triton X-100 last, mixing gently to avoid foaming.*

1. Place about 20 mL of distilled water in the beaker.
2. Add the calculated volume of 1 M Tris-HCl (pH 8) and mix.
3. Weigh out and dissolve the NaCl in the beaker; mix until completely dissolved.
4. Add the calculated volume of 160 mM EDTA with a micropipette.
5. Weigh out and dissolve the $MgCl_2$ in the beaker; mix until completely dissolved.
6. Add the glycerol (100%), measuring the volume with the pipette; mix slowly.
7. Add the Triton X-100 (10% stock), mixing gently without excessive agitation.
8. Transfer to the graduated cylinder and bring to volume with distilled water.
9. Keep the buffer on ice until use.

Steps from 2 to 7 not necessarily in this order

2. Protein Extraction from the Cell Pellet

Starting from the cell pellet, add the lysis buffer to disrupt the membranes and release intracellular proteins.

◇ **Buffer/cell ratio**

Use **1 mL of lysis buffer for every 5×10⁶ cells.** This ratio ensures that the proteins are sufficiently concentrated for subsequent analyses (e.g., Western blot, BCA/Bradford protein assay).

CALCULATIONS:

Number of cells in your sample: _____ cells

Volume of lysis buffer required:

V buffer = N° cells / (5×10⁶ cells/mL) = _____ / 5,000,000 = _____ mL

Volume of Protease Inhibitor Cocktail to add (1:100):

V Protease Inhibitor Cocktail (PI) = V *buffer* / 100 = _____ / 100 = _____ µL

Before adding the buffer to the pellet, add PI 1:100 (____ µL per mL of buffer).

⚠ DO NOT prepare this buffer in advance: always add the Protease Inhibitor Cocktail fresh at the time of use.

Procedure — *Balance all centrifuge tubes before spinning; keep everything on ice.*

10. Prepare the lysis buffer on ice and add the Protease Inhibitor Cocktail (10 µL/mL); mix briefly.
11. Aspirate the residual supernatant from the cell pellet without disturbing it.
12. Add the calculated volume of lysis buffer + PI directly onto the pellet.
13. Pipette the mixture up and down with the micropipette (at least 10 times) to resuspend the pellet.
14. Homogenise the sample with the microcentrifuge pestle, rotating gently until the visible pellet has completely dissolved.
15. Incubate the suspension on ice.
16. Centrifuge at 7500 g, 4°C, for 10 minutes. [Important: balance the tubes before inserting them in the rotor].
17. With a micropipette, transfer the supernatant (clarified lysate) to a clean, cold Eppendorf tube, avoiding contact with the pellet.
18. Store the lysate at −20°C until analysis.

Question: *After centrifugation, the pellet contains mainly: (a) soluble proteins, (b) nuclei and cell debris, (c) RNA. Which answer is correct? Justify your choice.*

Answer: _____

_____

Question: *Why is it essential to always work at 4°C or on ice during protein extraction? What would happen to the proteins at room temperature?*

Answer: _____

_____

Question: *The Protease Inhibitor Cocktail must be added FRESH at the time of use. Why can't you prepare the complete lysis buffer in advance and store it with the PI already added?*

Answer: _____

_____