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# Comparison of different protocols for protein extraction from murine ovarian tissue: A preliminary study

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## Abstract

**Objective***:* Extraction of proteins from tissues is a key step to study the protein structure and function. There is no set of universal protocols because of the enormous chemical and physical variety of both proteins and the sample sources. Hence, in this study a comparison of different methods for protein extraction from mouse ovarian tissue was carried out.

**Methods***:* Intravaginal dose of 20µl of Phosphate buffer saline was given to 7 mice for ten consecutive days followed by extraction of ovaries. For comparison, seven different protein extraction protocols from Protocol 1 to Protocol 7 were used. The supernatant was first quantified with Bradford assay to determine the total protein yield followed by SDS-PAGE analysis.

**Results***:* Highest protein yield of 1.13 mg/ml was attained after supplementing 1% v/v protease inhibitor cocktail in PBS. In total, 13 protein bands were detected by the extraction protocol 2 and 11 protein bands by the extraction protocol 1. 11 protein bands were detected in the range 180 kDa to 22 kDa following Protocol 3 and 4. Extraction with lysis buffer 1 resulted in 7 visible protein bands of molecular weight 182 kDa to 35 kDa range. Only 6 protein bands were observed in the range 182 kDa - 35 kDa following lysis buffer 2 method. For lysis buffer 3, ten visible protein bands were detected in range of 195 kDa - 13 kDa.

**Conclusion***:* Based on the protein extraction efficiency of Protocol 1, optimal combination of Phosphate buffer saline (pH 7.2) and protease inhibitor cocktail followed by hand-homogenization is suggested for protein extraction from mouse tissues.

**Keywords:** Protein extraction; Ovarian tissue; Homogenization; Lysis buffer

## 1. Introduction

Proteins are the end points of all biological functions, thus, represent the actual cell conditions. Protein profiling is the extensive study of proteins, particularly their structural and functional properties [1]. Basic protein profiling studies help in exploration of proteins present in a particular tissue. New biomarkers of disease can be found by comparative profiling of proteins in a healthy and diseased state at the same time.

Extraction of proteins from tissues or cell cultures is the first and key step for many biochemical and analytical techniques to study the protein structure and function and in protein purification. Protein extraction is the process to isolate and process the proteins from samples of whole tissues, cell cultures or biological fluids. The protein extraction protocol is usually tailored according to the starting material of the experiment and the end goals [2].

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Successful tissue protein extraction needs efficient cell lysis and homogenous tissue protein extraction while preserving protein's integrity and immunoreactivity. The algorithm of effective isolation and purification of protein could significantly vary as per its subunit structure, amino acid composition, net charge, isoelectric point, heat stability and hydrophobicity.

There is no set of universal protocols because of the enormous chemical and physical variety of both proteins and the sample sources in which they are found. Body fluids like urine and plasma, on the other hand, are already essentially homogenous protein solutions and only need to be collected. On the other hand, in order to disrupt tissue architecture, manage enzymatic activity, and solubilize the proteins in tissue samples, greater manipulations are necessary.

To extract proteins from tissues, homogenization and cell disruption methods can be categorized into mechanical/ sonication/ gentle lysis methods. Kang *et al* [3] states that prior investigation of protein methodologies is essential for designing an efficient and accurate proteomic workflow, tailored to the sample type. This process can optimize experimental design, reduce time, and cost, and improve the reproducibility and reliability of results.

As the critical step in the protein profiling studies is the extraction of proteins from samples, hence, in this study a comparison of different methods for protein extraction from mouse ovarian tissue was carried out. Optimization for murine ovary tissue was selected as it can be further explored to study reproductive disorders for protein profiling studies for analyzing comparative proteomics in diseased state.

## 2. Material and methods

### 2.1. Animal and Ethics

The study used seven sexually mature female BALB/c mice, aged 4-5 weeks, and weighing 22 ± 2g. The mice were kept under standard laboratory conditions with 12 hours of light and 12 hours of darkness, and were housed in cages with clean bedding in a well-ventilated animal room at the Department of Microbiology, Panjab University, Chandigarh. All mice had access to a standard pellet diet and water *ad libitum*.

### 2.2. PBS administration

Phosphate buffer saline (PBS, pH 7.2, 50mM) was prepared and used for inoculation. 7 mice were administered with 20µl of PBS for ten consecutive days.The conscious mice were manually restrained by holding its tail and held in a supine position with the posterior end slightly elevated and the head tilted lower than the body. The conscious mice were manually restrained by holding its tail and held in a supine position with its posterior end slightly elevated and the head tilted lower than the body. The mice were manually restrained by holding their tail, placed in a supine position with their posterior end slightly elevated and head tilted lower than the body while conscious. A dose consisting of 20 µl PBS was administered 1-2 mm deep into its vaginal opening with the help of an autopipette. The mouse was held in the same position till the liquid was fully absorbed. This process was repeated for each of the 7 mice for 10 consecutive days.

### 2.3. Extraction of ovaries from the mice

The mice were sacrificed by cervical dislocation and were carefully placed down on their back on an appropriate smooth wooden surface, then its limbs were spread and held firmly with pins simultaneously fixed in all four paws of the mice. The whole abdominal surface was gently and thoroughly sterilized with the help of 80% ethanol. The mice were dissected using a dissecting kit and the abdominal surface in the lower quadrant of the abdominal cavity was opened up through a ventral midline incision. After the abdomen was cut open, the ovaries were carefully separated under a magnifying glass and stored in sterile vial.

### 2.4. Protein extraction from ovarian tissue

Seven different methods for protein extraction were used. Protocol 1 to Protocol 7 with reagents and extraction method used are given below:

- **Protocol 1:** Tissue homogenizer (hand-held) + Ice-cold PBS Buffer (50mM, pH 7.2)
- **Protocol 2:** Tissue homogenizer (hand-held) + Ice-cold PBS Buffer (50mM, pH 7.2) supplemented with 1% v/v protease inhibitor cocktail (Sigma-Aldrich)
  The samples were homogenized in PBS buffer to prepare 10% homogenate
- **Protocol 3:** Ultrasonication + ice-cold PBS Buffer (50mM, pH 7.2) + Three cycles at 30% power, 30s on/ 30s off

- **Protocol 4:** Ultrasonication + ice-cold PBS Buffer (50mM, pH 7.2) supplemented with 1% v/v protease inhibitor cocktail (Sigma-Aldrich) + Three cycles at 30% power, 30s on/ 30s off
  The samples were homogenized in PBS buffer (10% homogenate) using probe sonicator while the samples were maintained in an ice bath (4°C)
- **Protocol 5:** Lysis buffer 1: 6M Urea, 2M thiourea supplemented with 1% v/v protease inhibitor cocktail
- **Protocol 6:** Lysis buffer 2: (modified RIPA buffer) 50mM Tris-HCL pH 7.5, 0.5% sodium deoxycholate, 150mM NaCl, 2mM EDTA, 0.1% SDS, 1% Triton X100 supplemented with 1% v/v protease inhibitor cocktail
- **Protocol 7:** Lysis buffer 3: 6M Urea, 50mM Tris-HCl, pH-8.5 supplemented with 1% v/v protease inhibitor cocktail
  The mixture/ homogenate was centrifuged at 10,000 rpm at 4°C for 15 minutes to remove all the cell debris. The supernatant was collected in sterile vial and stored at -20°C till it was used for analysis by SDS-PAGE.

### 2.5. Estimation of protein concentration

For the protein quantitation, Bradford assay was used as described by Bradford, 1976 and refined by Thomas Spector, 1978. Protein concentration (µg/ml) was estimated from the absorbance at 595 nm using a calibration curve with BSA.

### 2.6. Comparison of different protein extraction protocols by sds-page

SDS-PAGE analysis was performed as follows: the samples were prepared by mixing with sample buffer (loading buffer) containing bromophenol blue as tracking dye and were kept in boiling water for 5 min. The prepared samples were loaded onto 12% SDS-PAGE gels. The chamber was then covered and the gel was run at a constant voltage of 50 V. As the sample entered the separating gel, the voltage was increased to 100 V. After the completion of run, the gel was visualized by 0.25% Coomassie brilliant blue (R-250). For this, gel was kept in staining solution for 20 min with gentle agitation and eventually destained in destaining solution until background of the gel is fully destained.

## 3. Results

### 3.1. Estimation of concentration of proteins extracted by different extraction protocols from murine ovarian tissue

To provide a comparative analysis of various protein extraction methods from murine ovarian tissue, 7 protein extraction protocols were used and estimation of protein concentration by Bradford method was performed. The concentration of samples obtained from protocol 1 to protocol 7 was calculated with the standard curve for BSA.

A protein yield of 0.8 mg/ml was obtained after hand-homogenization with ice-cold PBS buffer, whereas, 1.13 mg/ml was attained after supplementing 1% v/v protease inhibitor cocktail in PBS. Following ultrasonication, total protein amount of 0.70 mg/ml (Protocol 3) and 0.75 mg/ml (Protocol 4) was obtained. Low protein yield i.e., 0.13 mg/ml and 0.14 mg/ml was obtained following Protocol 5 (6M Urea, 2M thiourea supplemented with 1% v/v protease inhibitor cocktail) and Protocol 7 (6M Urea, 50mM Tris-HCl, pH-8.5 supplemented with 1% v/v protease inhibitor cocktail), respectively. Extraction with Lysis buffer 2 (modified RIPA buffer) resulted in protein amount of 0.65 mg/ml.

Consequently, protocol 2 outperformed the other protocols where the hand-homogenization was performed with PBS supplemented with 1% v/v protease inhibitor cocktail. Further, the differences of extracted proteins were visualized by SDS-PAGE and all seven methods were compared.

### 3.2. Comparison of protein extracted by different extraction protocols by SDS-PAGE

After finding out the protein concentration in the samples extracted by different methods, protein profiling studies were carried out. The protein concentration was loaded in equal amounts ranging from 15 to 20 µg of protein extracts on SDS-PAGE gel and the results are as follows:

### 3.3. SDS-PAGE analysis of proteins of ovarian tissue extracted by Protocol 1 and Protocol 2

After proper destaining of the gel, protein bands of molecular weight 182 kDa, 157 kDa, 130 kDa, 100 kDa, 88 kDA, 69 kDa, 56 kDa, 45 kDa, 36 kDa, 32 kDa, 25 kDa were visibly observed in the sample extracted by PBS supplemented with 1% v/v protease inhibitor cocktail (hand homogenized) method and only PBS (hand-homogenized) method i.e., lane 1, lane 2 respectively.

Unlike lane 1, protein bands of 17 kDa, 13 kDa (low molecular weight) were also observed in lane 2 where PBS was supplemented with 1% v/v protease inhibitor cocktail. In total, 13 protein bands were detected by the extraction protocol 2 and 11 protein bands by the extraction protocol 1. Most of the visible bands were of high intensity in both methods. The protein bands for PBS (hand homogenized) and PBS + PIC (hand homogenized) method is represented in figure 1.

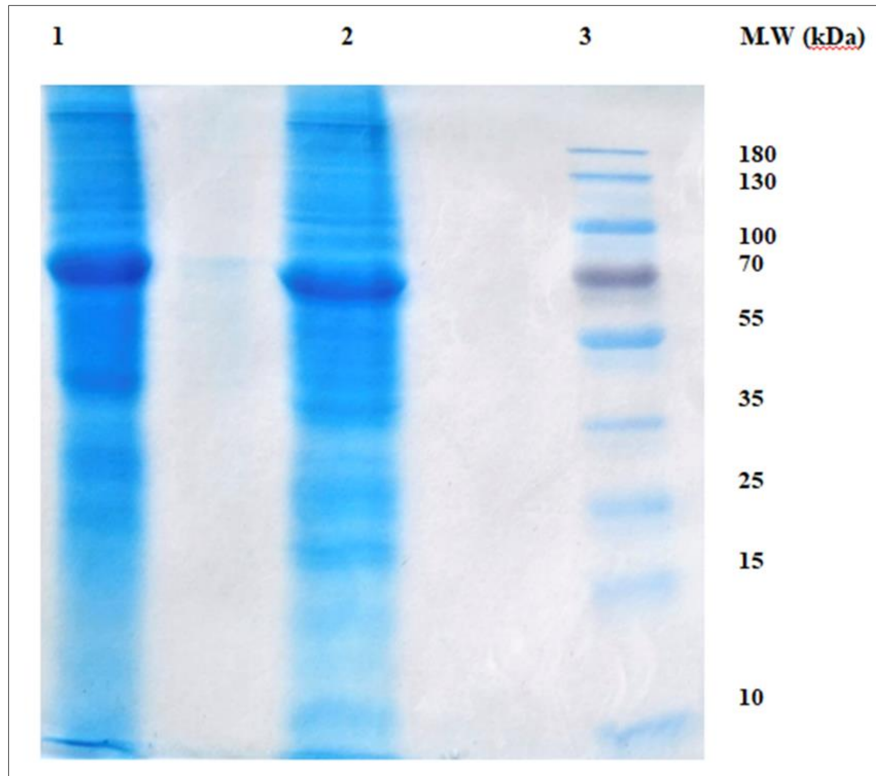


**Figure 1** Electrophoregram protein profile of ovarian tissue by Protocol 1 and Protocol 2 extraction method

Lane 1: ice-cold PBS Buffer (50mM, pH 7.2) + Hand-homogenization

Lane2: ice-cold PBS Buffer (50mM, pH 7.2) supplemented with 1% v/v protease inhibitor cocktail+ Hand-homogenization

Lane 3: Molecular weight marker

### 3.4. SDS-PAGE analysis of proteins of ovarian tissue extracted by Protocol 3 and Protocol 4

The, protein bands of molecular weight 180 kDa, 130 kDa, 100 kDa, 88 kDA, 69 kDa, 56 kDa, 45 kDa, 37 kDa, 35 kDa, 28 kDa and 22 kDa were visibly observed both in the sample extracted by PBS (Ultra-sonication) and PBS supplemented with 1% v/v protease inhibitor cocktail (Ultra-sonication) method i.e., lane 2 and 3. However, 69 kDa 37 kDa, 28 kDa and 22 kDa protein bands were of high intensity in protocol 4 where PBS was supplemented with 1% v/v protease inhibitor cocktail (Figure 2).

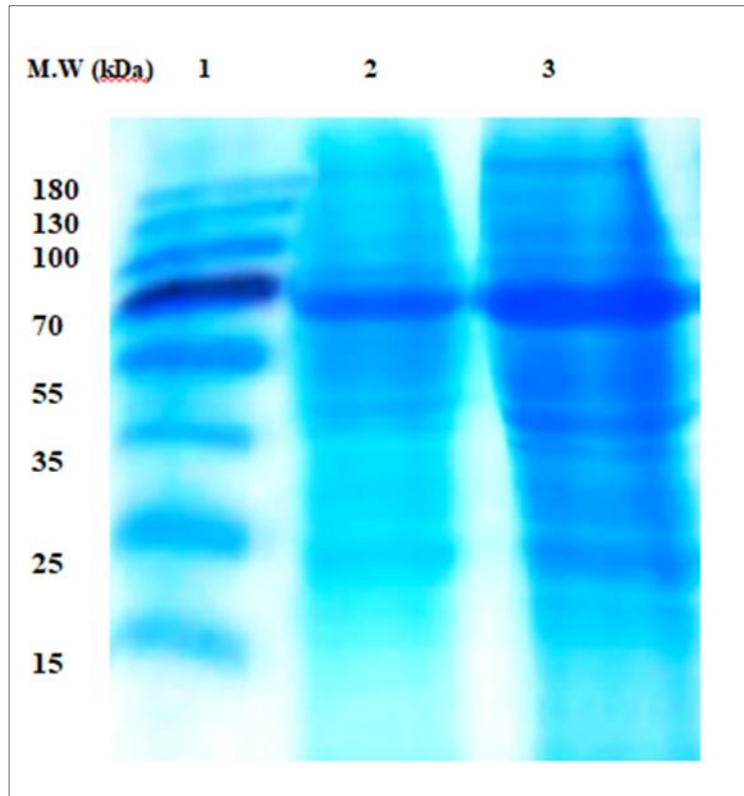


**Figure 2** Electrophoregram protein profile of ovarian tissue by Protocol 3 and Protocol 4 extraction method

Lane 1: Molecular weight marker

Lane2: ice-cold PBS Buffer (50mM, pH 7.2) + Ultra-sonication

Lane 3: ice-cold PBS Buffer (50mM, pH 7.2) supplemented with 1% v/v protease inhibitor cocktail (Sigma-Aldrich) + Ultra-sonication

### 3.5. SDS-PAGE analysis of proteins of murine ovarian tissue extracted by Protocol 5-7 (Lysis buffer)

Extraction with lysis buffer 1 (6M Urea, 2M thiourea supplemented with 1% v/v protease inhibitor cocktail) resulted in bands of ~~of~~ molecular weight 182kDa, 130 kDa, 100kDa, 70kDa, 55kDa, 38kDa and 35 kDa (Figure 3, Lane 1).

Protein bands of molecular weight 182kDa, 130 kDa, 100kDa, 70kDa, 55kDa and 35 kDa were visible in Lysis buffer 2 (modified RIPA buffer: 50mM Tris-HCL pH 7.5, 0.5% sodium deoxycholate, 150mM NaCl, 2mM EDTA, 0.1 SDS, 1% Triton X100 supplemented with 1% v/v protease inhibitor cocktail) method (Figure 3, Lane 3).

Protein bands of molecular weight 182 kDa, 155 kDa, 130 kDa, 100 kDa, 69 kDa, 47 kDa, 35 kDa, 27 kDa, 17 kDa and 13 kDa were detected on the gel after using lysis buffer 3 (6M Urea, 50mM Tris-HCl, pH-8.5 supplemented with 1% v/v protease inhibitor cocktail) (Figure 4).

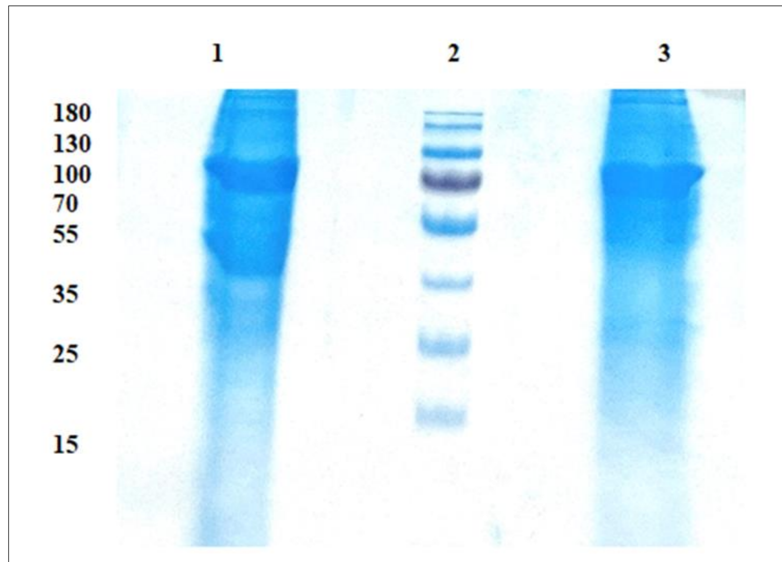


**Figure 3** Electrophoregram protein profile of murine ovarian tissue extracted by using Lysis buffer 1 and Lysis buffer 2 methods

Lane 1: Lysis Buffer 1 (6M Urea, 2M thiourea supplemented with 1% v/v protease inhibitor cocktail)

Lane 2: M.W marker

Lane 3: Lysis Buffer 2 (modified RIPA buffer) (50mM Tris-HCl pH 7.5, 0.5% sodium deoxycholate, 150mM NaCl, 2mM EDTA, 0.1% SDS, 1% Triton X-100 supplemented with 1% v/v protease inhibitor cocktail)

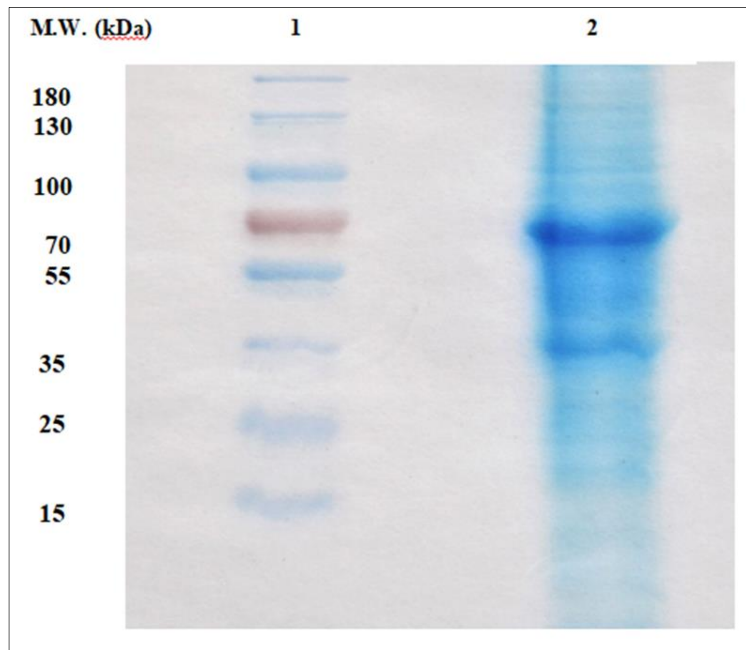


**Figure 4** Electrophoregram protein profile of murine ovarian tissue extracted by Lysis buffer 3 method

Lane 1: Molecular weight marker

Lane 2: Lysis Buffer 3 (6M Urea, 50mM Tris-HCl, pH-8.5 supplemented with 1% v/v protease inhibitor cocktail).

## 4. Discussion

Due to the varied properties of proteins, there is no single protocol for protein extraction that can be universally applied. The choice of buffer can significantly impact the yield of protein extracted from different tissues, making it essential to optimize the buffer type and additives to obtain a better yield of total protein.

This study aimed to compare the total protein yield of seven different protein extraction protocols. The protein extraction efficiency of seven protein extraction methods was compared after Bradford assay. A protein yield of 0.8 mg/ml was obtained after hand-homogenization with ice-cold PBS buffer, whereas, 1.13 mg/ml was attained after supplementing 1% v/v protease inhibitor cocktail in PBS. In total, 13 protein bands were detected by the extraction protocol 2 and 11 protein bands by the extraction protocol 1. Protein bands of 17 kDa, 13 kDa (low molecular weight) were observed where PBS was supplemented with protease inhibitor cocktail.

The study found that the total protein concentration and protein bands were significantly higher in the PBS buffer containing protease inhibitor cocktail (PIC) as compared to PBS buffer alone. The absence of a protease inhibitor in the buffer can cause proteins to be more susceptible to degradation, leading to a lower total protein yield due to the release of enzymes from the cell compartment after cell disruption. Protease inhibitors are important as they protect the protein samples from being degraded by proteases that can be liberated from the membrane and cellular compartments [4]. Additionally, the presence of sodium chloride in the buffer helps to keep the proteins soluble and increases the ionic strength of the buffer, which facilitates the disruption of molecular interactions.

Following ultrasonication, total protein amount of 0.70 mg/ml (Protocol 3) and 0.75 mg/ml (Protocol 4) was obtained, i.e., relatively lower than obtained after hand-homogenization method. 11 protein bands were detected in the range 180 kDa to 22 kDa in both the above methods.However, 69 kDa 37 kDa, 28 kDa and 22 kDa protein bands were of high intensity in protocol 4 where PBS was supplemented with protease inhibitor cocktail. In accordance to this, Sethi *et al* [5] have employed ultrasonication method during tissue lysis of mouse brain lysates. However, a major disadvantage associated with this method is that it produces heat which may result in damage to the sensitive proteins.

According to some studies, RIPA buffer is a popular choice for protein extraction from mammalian tissue [6] as it contains dilute sodium dodecyl sulfate (SDS), which is a denaturing detergent, and deoxycholate, which can disrupt protein-protein interactions. RIPA buffer is commonly used as a lysis buffer for whole-cell extraction due to its effectiveness in breaking down cells and extracting their protein content [7].

In the present study, extraction with Lysis buffer 2 (modified RIPA buffer) resulted in protein amount of 0.65 mg/ml, relatively higher than other lysis buffer used. The substantial increase in protein yield observed may be attributed to the inclusion of detergents such as Triton X-100, sodium deoxycholate, and sodium dodecyl sulfate, which aid in solubilizing poorly soluble proteins and disrupting the membrane structure of the cell. In contrast to this, following this method, only 6 protein bands were observed in the range 182 kDa - 35 kDa. Thermo Fisher Scientific has reported that one drawback of this detergent formulation is its comparatively lower compatibility with certain downstream applications when compared to other lysis reagents. Henceforth, the lower protein bands can be attributed to poor compatibility of RIPA buffer to gel electrophoresis.

Urea-based buffers have been reported to be widely used in tissue proteomics [8, 9], therefore we tested the extraction buffer containing urea in Protocol 5 and 7. Contrary to the previous studies, low protein yield i.e., 0.13 mg/ml and 0.14 mg/ml was obtained following Protocol 5 and Protocol 7, respectively. Extraction with lysis buffer 1 resulted in 7 visible protein bands of molecular weight 182kDa to 35 kDa range.

For lysis buffer 3, ten visible protein bands were detected in range of 182 kDa - 13 kDa, where urea along with Tris-HCl (pH-8.5) was used. Several studies have reported that alkaline Tris-HCl buffers with a pH range of 8.0–9.5 are more effective in producing high-quality protein extractions compared to neutral or acidic buffers [10]. These variations in protein yield may be attributed to the different compositions of buffers utilized, which can lead to varied cell disruptions and subsequently influence the release of proteins from cell compartments.

Dowling et al. [11] propose that during the initial preparation of tissue extracts, the addition of an appropriate protease inhibitor cocktail to the buffers is essential to prevent the degradation of susceptible proteins. Consequently, protocol 2 outperformed the other protocols where the hand-homogenization was performed with PBS supplemented with protease inhibitor cocktail.

## 5. Conclusion

Based on the above findings, our study concludes that, for efficient protein extraction from murine tissues, buffers should be supplemented with a suitable protease inhibitor cocktail. Based on the protein extraction efficiency of Protocol 2, optimal combination of Phosphate buffer saline (pH 7.2) and protease inhibitor cocktail followed by hand-homogenization is suggested for protein extraction from mouse tissues.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest.

### *Statement of ethical approval*

The prospective study procedures were approved by Institutional Animal Ethics Committee, Panjab University; Approval No. PU/45/99/CPCSEA/IAEC/2021/658 and the experiments were carried out ensuing the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals. Ovaries were donated by Thomson soni.

## References


[1] Chandramouli K, and Qian PY. Proteomics: challenges, techniques and possibilities to overcome biological sample complexity. Human genomics and proteomics: HGP 2009 Dec;1-22.

[2] Lee CH. A simple outline of methods for protein isolation and purification. Endocrinology and Metabolism. 2017 Mar;32(1):18-22.

[3] Kang L, Weng N, Jian W. LC–MS bioanalysis of intact proteins and peptides. Biomedical chromatography. 2020 Jan;34(1):e4633.

[4] G-Biosciences. Protease and Phosphatase Inhibitors, Enzymes and Assays Handbook and Selection Guide. G-Biosciences. 2018.

[5] Sethi MK, Downs M, Zaia J. Serial in-solution digestion protocol for mass spectrometry-based glycomics and proteomics analysis. Molecular Omics. 2020 Aug 1;16(4):364-376.

[6] Kurien BT, Scofield RH. Western blotting: an introduction. Western Blotting. Methods in Molecular Biology 2015; 1312:17-30.

[7] Ngoka L. Sample prep for proteomics of breast cancer: proteomics and gene ontology reveal dramatic differences in protein solubilization preferences of radioimmunoprecipitation assay and urea lysis buffers. Proteome science. 2008 Oct 24;6:30.

[8] Rodríguez-Rigueiro T, Valladares-Ayerbes M, Haz-Conde M, et al. A novel procedure for protein extraction from formalin-fixed paraffin-embedded tissues. 2011 Jun;11(12):2555-9.

[9] Guo T, Kouvonen P, Koh CC, et al. Rapid mass spectrometric conversion of tissue biopsy samples into permanent quantitative digital proteome maps. Nature medicine. 015 Apr;21(4):407-13.

[10] Chung JY, Lee SJ, Kris Y, et al. A well-based reverse-phase protein array applicable to extracts from formalin-fixed paraffin-embedded tissue. Methods in Molecular Biology. 2015;1312:129-39.

[11] Dowling P, Gargan S, Zweyer M, et al. Proteome-wide changes in the mdx-4cv spleen due to pathophysiological cross talk with dystrophin-deficient skeletal muscle. Iscience. 2020 Aug 26;23(9):101500.