Sample lysis instructions
VERSION FOR HARD TISSUE

1. Introduction

Proteome Sciences cannot receive infectious agents or genetically modified organisms. Infectious agents or genetically modified organisms **must** be inactivated and processed to a **cell-free protein solution** prior to shipment. Sample lysis and buffer protocols to achieve a cell-free protein solution are provided below. Please note that our recommended lysis buffers are workflow dependent. If you require guidance as to which lysis buffer you should use for your study please contact us (contact details are provided below).

This document describes the protocol and lysis buffers recommended for the preparation of cell-free protein solutions from hard tissues (e.g. liver, muscle, skin). For sample lysis instructions for other sample types please contact us. Generally, masses of tissue or number of cells needed to achieve protein masses needed vary significantly depending on tissue and cells and have to be determined individually. A rough first guidance is that for many tissues ~5% of the tissue wet weight can be extracted as protein and that from a typical mammalian cell ~100 pg of protein can be extracted when processing pellets in the ~10^7-10^8 range (e.g. in a project where we had 10^6 of a human cell line, this delivered sufficient protein material for a TMT® MS2/MS3 study; for a SysQuant® study cell numbers usually need to be in the higher 10^6 or 10^7 numbers). For a TMT®MS2 workflow, lysis of for example ~20 milligrams of tissue in for example 100 microliters of SysQuant® lysis buffer, typically will easily exceed sufficient total protein for a study and in most cases suffice also for a SysQuant® study. For the protein mass needed for the selected workflow in your project case refer to the proposal document or contact us. This will determine the minimal input masses for tissue or number of cells.

We recommend production of backup samples in separate vials that can be used in case that some of the original samples do not deliver satisfactory amounts or protein band patterns.

2. Lysis buffer selection

Our recommended lysis buffers for the preparation of cell-free protein solutions from hard tissues are product workflow dependent. Customers are advised to consult their proposal document which will list their study product workflow and then select the most appropriate buffer from table one below. If the same tissue is to be used across multiple work packages it is recommended to split the frozen sample for the different lyses.

For immunoprecipitations we recommend lysis in RIPA-buffer or IP lysis buffer (see separate document). As the interactions of antibody, target protein and co-immunoprecipitates can be very case-specific and buffer-dependent, the respective buffer can be modified as to previous experience of the customer. The IP lysis buffer described below does not contain SDS and therefore is a milder version of a lysis buffer that e.g. should be better suited if analysis co-immunoprecipitates or protein complexes is aimed for.

If customers are unsure which buffer is most appropriate for their study they are requested to contact us.

Table 1 – Lysis buffer selection

Product Workflow	**Required lysis buffer**	**Comment on protein mass per sample**
SysQuant®	SysQuant® lysis buffer	Ideal 1mg, but can work with lower like e.g. 300-400 µg
TMT®MS2	SysQuant® lysis buffer	Aim for ≥100 µg, but can work lower
TMT®MS3	SysQuant® lysis buffer	Aim for ≥100 µg, but can work lower
Immunopreciptation(IP)-MS	RIPA-buffer or IP-buffer	Contact us
Bespoke Assay Development	Contact us	Contact us
Other	Contact us	Contact us

3. Reagents, Material and Instruments:

Reagents

- Complete Mini (Roche, cat. no. 04 693 124 001), 30 tablets. Roche.
- Sodium chloride (Applichem, cat. no. A1371,1000)
- PhosSTOP (Roche, cat. no. 04 906 845 001), 10 tablets
- Tris hydrochloride (Applichem, A3452,0250)
- Urea (Merck, cat. no. 108487.1000)
- Ultrapure water

- If reagents are not available they can be replaced by reagents of comparable quality from different providers.

Material

- Protein Assay Dye Reagent Concentrate (Bio-Rad, 500-0006)
- BSA (for standard curve in protein assay) (Bio-Rad, 5000208)
- IgG (for standard curve in protein assay) (Bio-Rad, 5000206)

Instruments

- Centrifuge for microtubes (e.g. Eppendorf 5417R, Inv.-Nr.: 3000046.00 or 3000136.00)
- Incubator for microtubes
- Sonifier (Branson, W450D, Inv.-Nr.: 3000154.00)
- Vortex Shaker
- Scales for weighing out tissue and tissue powder (Mettler MS204, Inv.-Nr.: 3000422.00)

Reagent setup

- **SysQuant lysis buffer for mammalian cells or tissues**
  8 M urea, 75 mM NaCl, 50 mM Tris, pH 8.2, one tablet of protease inhibitors cocktail (complete mini, Roche) and one tablet of phosphatase inhibitor cocktail (Roche) per 10 mL of SysQuant lysis buffer.

Critical: It is recommended to prepare the buffer fresh before use.

Comment on buffer preparation:

Note that solving urea to 8M in 75 mM NaCl and 50 mM Tris takes time. As 8M Urea is a very high concentration, the volume of the buffer solution will be higher than the water volume added. We often use ~2/3 of the volume intended for the end volume and fill up later, after dissolving. When dissolving the urea powder (and NaCl) in bidest the solution will initially become cold to the touch. Warm the bottle at 20–25°C for ~30 minutes or longer, while mixing periodically to ensure complete dissolution. A 30°C water bath will aid in the dissolution of the powdered urea, also a short warming in the microwave is ok. It is recommended not allow the temperature of the solution to rise above 30°C, since cyanates may begin to form that will be detrimental to the proteins. Then add Tris-HCl, NaCl, (check pH, eventually adjust to 8.2), add protease inhibitors, phosphatase inhibitors, fill up with water to planned ml. Cool down in the refrigerator to 4°C. It is possible to prepare the base buffer with urea, NaCl, tris before, store at 4°C and add protease and phosphatase inhibitors freshly before use.

It is not recommended to freeze the buffer because precipitates will form.

Risks:	Wear lab coat, safety glasses and gloves. Pay attention to safety instructions of manufacturers.

Procedure:

4. Tissue lysis

The tissue lysis protocol is dependent on the type of tissue used. The lysis steps, volumes etc. may vary significantly. Tissue samples from organs not used before need to be tested to determine procedure and yields from available material. Tissues are often categorized as soft tissues or hard tissues. Typically used procedures are described below for fresh frozen tissue. Protein recoveries are often in the range of 5-10% of protein mass from tissue wet weight.

4.1. **Hard tissues (e.g. liver, pancreas or breast cancer tissue)**

4.1.1. All steps using frozen tissues are carried out with cooling in liquid nitrogen in order to ensure that the tissue stays frozen.

4.1.2. Hard tissues need to be pulverized before being lysed in SysQuant lysis buffer (see above). The yields are often around 50-100 µg of total protein per mg of available tissue.

4.1.3. Weigh out the masses of the tissues and make a record of these.

4.1.4. Determine if tissue amount is expected to deliver the desired amount of protein for each of the samples and discuss with project manager if tissues need to be aliquoted.

4.1.5. Tissues with masses between ~10-100 mg are pulverized using a MultiSample BioPulveriser (MSBP, Biospec, Cat. No. 59012N). Tissues with amounts over 100 mg need either to be broken into smaller portions to be pulverized or if more than 100 mg need to be pulverized using the Dismembrator and teflon capsula (Braun, Melsungen) under liquid nitrogen. This procedure is not described here in detail but can be adapted from the classical lysis for TMT-workflows.

4.1.6. Other methods to homogenize the hard tissue in frozen state are equally useable (ball mills, etc.)

4.1.7. The BioPulverizer consists of a hole machined in a stainless steel base into which fits a special piston, or pestle. In summary, a typical procedure involves

tissue being maintained in the hard-frozen state in liquid nitrogen and placed in the pre-chilled BioPulverizer. The piston delivers a blow to the brittle tissue reducing it to powder.

4.1.8. Place the 12 stainless steel sleeves, rubber 0-ring ends facing up, in the shallow wells of the MSBP base. While pressing the release button, insert the T- handle into the center hole. Once inserted, release the button and you should now be able to lift the MSBP base.

4.1.9. Place the assembled MSBP and the pestle rods in a plastic tray and thoroughly chill them with liquid nitrogen or overnight in a -80^{o}C freezer (the latter being the easiest and preferred method). Stainless steel is surprisingly slow to cool, so if one requires cooling of the MSBP immediately liquid nitrogen cooling is preferable. Once cold, the MSBP holds its low temperature for almost an hour. Be sure the MSBP is cooled completely. It takes about 1-2 cups of liquid nitrogen to complete the cooling process. Start by slowly pouring liquid nitrogen over the stainless steel parts. As first the liquid nitrogen will flash off as vapour. After a minute or so of bathing with liquid nitrogen all parts should be cold enough and the MSBP may even retain a few puddles of liquid nitrogen in the wells. There is no need to add more liquid nitrogen.

4.1.10. Add appropriate sized pre-frozen tissue to the wells of the mortar (fresh tissue sticks to the cold metal and cannot be inserted into the wells of the MSBP).

4.1.11. Remove the MSBP from the plastic tray and set it on the lab bench. On the T-handle, press the release button, lift the T-handle from the MSBP base, and set the T-handle aside. You should have plenty of time to process the samples without the need to re-cool the stainless steel MSBP.

4.1.12. Insert the chilled pestle into a sleeve of the mortar (Caution: Use gloves or mittens. Very cold metal parts can inflict skin “burns”) and deliver two or three sharp blows to the pestle using a hammer.

4.1.13. In the ideal case the powdered tissue will be in the form of a compacted pellet lodged in the bottom part of the sleeve of the MSBP. Transfer the frozen tissue powder into a chilled Eppendorf vial using a chilled spatula. Keep the tissue powder frozen on dry ice until all tissue samples are pulverized.

4.1.14. Lysis of the tissue powder: Add chilled SysQuant lysis buffer (~4°C) to the frozen tissue powder (e.g. per 20 mg liver tissue powder in ~0.4 ml cold buffer resulted in 2 mg of total protein ~5 µg/µl in 0.4 ml). Ensure powderized tissue completely dissolved in buffer.

4.1.15. Sonication 10-20% amplitude for 20 x 1 second, pulsing on and off, on ice (4°C). Repeat if necessary. Often at least three repeat cycles are needed. It is recommended to gradually increase amplitude from 10% if needed up to 20%. CAUTION: When immediately using higher amplitudes the fluid may spill therefore care is required.

4.1.16. Centrifuge at 12,500g for 10 min at 4°C to eliminate tissue debris and transfer the supernatant (cell-free protein solution) into new tubes.

4.1.17. Optional: check lysis microscopically.

4.1.18. Measure protein concentration using Bradford assay (using a standard curve mixture of BSA and IGG).

4.1.19. After determining protein concentration, aliquot suitable protein masses from each sample into a clean fresh Eppendorf tube.

4.1.20. Quality control: apply e.g. 5µg of each sample to a 1-D SDS-PAGE gel and stain with Coomassie.

4.1.21. Store cell-free protein solutions (samples) at -80°C.

5 Sample Storage & Shipment

Following sample lysis and prior to shipment sample should be stored at -80°C.

Customers are advised to refer to our sample shipping instruction sheet (IF-3.10.22) for further instructions on shipment of infectious agents or genetically modified organisms including customs requirements.

6 PS Contact Details

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