Proteome Sciences R&D GmbH & Co. KG - Strictly confidential

Sample lysis instructions
VERSION FOR CELL PELLETS

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 cell pellets. 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 cells 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 system (ELGA Purelab Plus UV/UF (Inv.-Nr. 300255.00)), ELGA Purelab Classic UV/UF (Inv.-Nr. 30000361.00)

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

- 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

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 takes time. As 8M Urea is a very high concentration, the volume of the buffer solution will be higher than the water volume added. 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 volume. 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. Cell lysis

NOTE 1: The protein extracted per mammalian cell depends very much on the cell type. But as a rough guide 50-150 pg/cell at cell number ranges of a few million in handling can be taken. In case of cell pellets the respective amount of lysis buffer is estimated from the number of washed cells in the pellet or dish to reach a rough concentration of e.g.~10 mg/ml and added to produce a suspension to be used for lysis through sonication. However, if e.g. volumes would be too low for efficient use in the lysis/sonicationwith sonication tips the volumes can be scaled up. In such cases good results were e.g. produced with 250 µl per 10^7 cells or 100 µl per $2x10^6$ cells where then sonication in sonication bath was used.

NOTE 2: For non-adherent cells it is recommended to produce a washed cell pellet in Eppendorf tubes and then add appropriate volume of lysis buffer (if lysis is planned, otherwise pellets can be flash frozen and stored at -80°C before processing or shipment). Proper washing is important to remove medium components that often contain e.g. BSA.

NOTE 3: For adherent cells: proper washing is important to remove medium components that often contain e.g. BSA. If a method for gentle detachment is known then it is recommended to produce a washed cell pellet in Eppendorf tubes for storage or shipment. In case that lysis is to be performed add appropriate volume of lysis buffer. If no appropriate method for cell detachment is known then perform washing and lysis on dishes directly (separate document).

4.1. Wash cells with appropriate volume of sterile-PBS and centrifuge at appropriate conditions. Repeat this step e.g three times.

4.2. Aspirate PBS and add chilled lysis buffer to the cell pellet (suspend pellet appropriately). A typical example: for $1x10^7$ cells we often use 250 µl of SysQuant lysis buffer.

4.3. Pause point: If samples shall be stored then pellets need to be flash-frozen and stored at -80°C before further processing or shipment to PS.

4.4. Sonication 10% amplitude for 20 x 1 second, pulsing on and off, on ice (4°C). Repeat and increase amplitude to 15 or 20% if necessary. Usually cells disintegrate within a few cycles. In most cases the amplitude does not have to be higher than 10%. CAUTION: When immediately using higher amplitudes the fluid may spill therefore care is required.

4.5. Note: if a sonciation tip is not available or volumes are not appropriate for the use of the available tips then lysis can also be performed using a sonciation bath using ice water, conditions need to be set-up and can depend on cells and volumes.

4.6. Centrifuge at 12,500g for 10 min at 4°C to eliminate cell debris and transfer the supernatant into new tubes.

4.7. Measure protein concentration using modified Bradford assay (BioRad Protein Assay) against a 1:1 standard curve of IgG/BSA. Basically, we use the microassay procedure in point 2.3 of the attached manufacturer instruction to determine the protein concentration. Calculate total protein mass (volume x concentration).

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

4.9. 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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