Preparation of a tissue lysate

Courtesy Tieneke Schaaij-Visser

1. Keep the frozen tissue in liquid nitrogen/on dry ice until the last moment.

2. Prepare sufficient Reducing Sample Buffer (RSB, see below).

3. Create a clean, cold surface (cooled with dry ice) for cutting the tissue.
(*this can be done in several ways, depending on your liking: e.g., wrap a piece of dry ice in tin foil, or fill a tin vial (generally used for tissue storage in liquid nitrogen) with dry ice, and use it upside down as a little "cutting table"; a cleaned glass plate/slide on dry ice may also be used; some 'hoar frost' will inevitably develop*).

4. Cool a labelled 1.5-ml Eppendorf microcentrifuge tube, place it on a balance, tare (set reading to 0 with "0/T"), and return it to dry ice (or liquid nitrogen).

5. Use a clean and cold scalpel (immerse in liquid nitrogen) to cut off a small piece of the tissue: aim for 5-20 mg.

   The crushing procedure does not work well for smaller or larger pieces.

6. *Quickly* weigh the piece of tissue in the cooled microcentrifuge tube used to tare. Immediately return the tube to dry ice.

7. Calculate the required volume of Reducing Sample Buffer (RSB) to homogenise the pertinent piece of tissue.

   Use a ***proper buffer-to-tissue ratio*** for the tissue you are using; this may differ for different tissues, but a good starting point is 40 µl buffer per mg tissue (i.e. homogenise, e.g., 20 mg tissue in 800 µl RSB).

   If this was not established before, try a range of buffer-to-tissue ratios in a test experiment to determine optimal homogenisation conditions.

8. Cool the microcentrifuge tube containing the tissue piece as well as the tip of a plastic homogenisation pestle (e.g., a Kontes Pellet Pestle for 0.5-ml tubes*) by immersing them in liquid nitrogen.

*Kontes Pellet Pestle
NL: Fisher Scientific cat#6330288 - Pellet stamper 0,5 ml
(www.emergolab.com)*

9. Place the microcentrifuge tube firmly in a tube rack, and *quickly* crush the tissue with the cooled pestle until it has become a powder.

   Be careful, fragments may eject out of the tube!; if necessary use a tiny cover of tin foil or a tube lid through which you stick the pestle to make sure all tissue pieces stay in the tube.

10. Add the calculated amount of RSB, mix well using the pestle, and immediately heat the tube for 5-10 min at 99°C in a heating block.

11. Spin down particulates in a microcentrifuge (maximum speed) for 5-10 minutes at room temperature.

12. Transfer the supernatant (= lysate) to a new, properly labelled microcentrifuge tube.

13. If there is a big pellet, you can add another 25-50 µl of RSB to it, homogenise with the pestle, and spin down again. You should then pool the two lysates.

    If the lysate is too slimy after cooling down, you can increase the amount of RSB, or cook the sample a second time.

    After heating the lysate, you can keep it at room temperature if you will use it immediately, or make aliquots and store them at -80°C.

    Always heat the lysate and spin particulates down before loading it on gel.

14. On an SDS-PAGE gel, load a range of sample volumes such as 5, 10 and 20 µl, to check which volume is the best for, e.g., in-gel digestion purposes.

    The aim is to load the maximum without protein smearing along the lane (sign of overloading). In case of smearing, dilute the sample in RSB and heat again.

<u>Reducing Sample Buffer (RSB)</u>:

Dilute:

- 4x concentrated NuPAGE sample buffer (or 2x concentrated Laemmli sample buffer)
- 10x concentrated DTT (1M)

to a 1x concentration with double-distilled (milliQ) water to end up with 1x RSB.

<u>4x NuPAGE sample buffer</u>:

423 mM Tris HCl
563 mM Tris base
8% LDS
40% Glycerol
2.05 mM EDTA
0.88 mM SERVA® Blue G250
0.70 mM Phenol Red
(pH of 1x will be 8.5)

<u>2 x Laemmli sample buffer</u>:

125 m M Tris-HCl pH 6.8
4% SDS
20% glycerol
0.005% BFB