OptiPrep™ Application Sheet S05

Homogenization of mammalian tissues

1. Homogenization techniques

Mammalian tissues fall generally into two groups: soft tissues (e.g. rat liver) and hard tissues (e.g. bovine muscle) and routinely the types of homogenizer used to disrupt these tissues are liquid shear (Potter-Elvehjem or Dounce) or mechanical shear (e.g. Polytron) respectively. The situation is not clear-cut however since hard tissues may be rendered susceptible to liquid shear homogenization by treatment with hydrolytic enzymes. Whatever technique is used it is good practice to facilitate the homogenization by an initial coarse mincing of the tissue with scissors, scalpels or (for large masses of tissue) a mincer.

2. Removal of blood

Highly vascular tissues such as rat liver may require some form of perfusion to remove blood from the vasculature prior to homogenization. This is particularly true if the nuclear pellet is to be processed, for any erythrocytes in the homogenate will sediment at low g-forces. Erythrocytes may also interfere with the functional characterization of a particular organelle, for example the catalase in these cells may obscure any assessment of the fractionation of peroxisomes by measurements of this enzyme. Perfusion can be carried out after sacrificing the animal, simply by injection of buffered saline or homogenization medium through the portal vein after cutting the blood vessels above the liver. It is best carried out however under anaesthesia when the portal vein can be properly cannulated. This must be performed by a trained and licensed operative.

3. Homogenization media

Routinely, most **soft tissues** are homogenized in 0.25 M sucrose, buffered with low concentrations of an organic buffer such as Tris, Hepes or Tricine at a pH between 7 and 8. Often 1 mM EDTA is included to reduce aggregation, but if the organelle of interest is the nucleus, the EDTA is replaced with 25 mM KCl and 5 mM $MgCl_2$, while for sheets of plasma membrane use 1 mM $MgCl_2$. For mitochondria, the sucrose may be replaced by mannitol. Brain tissues are frequently disrupted in 0.32 M sucrose rather than 0.25 M.

Hypoosmotic media (e.g. 10 mM Tris-HCl, pH 7.5 or 5 mM EDTA, pH 7.4) are often used with intestinal mucosa [1] and 1 mM $NaHCO_3$ has been used for rat liver for the isolation of large sheets of plasma membrane although it is mow recognized that an isoosmotic medium can be just as effective [2].

Media for **muscle** homogenization are also quite variable and although compositions not unlike those for soft tissues have been used, KCl is often included (up to 180 mM) to solubilize some of the protein and prevent the formation of gels. The following media have been successfully used: 0.21 M mannitol, 70 mM sucrose, 0.1 mM EDTA, 0.5% bovine serum albumin (BSA), 10 mM Tris-HCl, pH 7.4 or 0.1 M sucrose, 10 mM EDTA, 46 mM KCl, 0.5% BSA, 100 mM Tris-HCl, pH 7.4 [3]. After coarse mincing of muscle tissue, it is commonly softened by incubating with Nagarse at 5-50 mg per 100ml at 4°C for about 5 min.

3. Homogenization of rat liver

3a. Equipment and solutions required

A. Potter-Elvehjem homogenizer (30-40 ml), clearance approx 0.08 mm
B. Wall mounted, high-torque, thyristor controlled electric motor
C. Muslin or nylon mesh (75 µm pore size)
D. Homogenization medium (HM): 0.25 M sucrose, 1 mM EDTA, 20 mM HEPES-NaOH, pH 7.4; for nuclei replace the EDTA with 5 mM $MgCl_2$ and 25 mM KCl (see Box on p. 2)

3b. Protocol

Keep all the equipment on ice.

Keep the following stock solutions at 4°C:
500 mM Hepes (free acid): 11.9 g per 100 ml water.
100 mM EDTA (Na_2•$2H_2O$): 3.72 g per 100 ml water
1 M KCl: 7.45 g per 100 ml water
1 M $MgCl_2$•$6H_2O$: 20.3 g per 100 ml water

Solution D: Dissolve 17 g sucrose in 100 ml water; add 2 ml of EDTA stock and 8 ml of Hepes stock; adjust to pH 7.4 with 1 M NaOH and make up to 200 ml.

For nuclei replace the EDTA with 1.0 ml of the $MgCl_2$ stock and 5.0 ml of the KCl stock

1. Perfuse the liver if necessary, and then rapidly excise the tissue into ice-cold HM.

2. Transfer the liver into a 50 ml beaker (on ice) and mince with scissors, the pieces of tissue should be no more than 30 mm^3.

3. For one liver (10-12 g) suspend the coarse mince in 40 ml of Solution D; stir and then decant the liquid after the mince has settled out. Repeat this process; finally suspend in 40 ml of Solution D and transfer half to the glass vessel of the homogenizer.

4. Secure the ice-cold pestle in the chuck of the electric motor and with the pestle rotating at 500-800 rpm homogenize the liver using 5-6 up-and-down strokes of the pestle. If the tissue becomes compacted at the bottom of the vessel; withdraw the pestle and allow the vortex action in the liquid to resuspend the tissue (see Notes 1-3).

5. Repeat the procedure with second half of the tissue suspension.

6. If required filter through nylon gauze or three layers of muslin to remove undisrupted cells and connective tissue. Do not force the suspension through the filter by squeezing.

4. Notes

1. This method can be used as a general-purpose homogenization procedure for the isolation of most organelles and membrane particles from most soft tissues or enzyme-digested hard tissues. See ref 4 for more information on homogenization techniques.
2. To isolate sheets of plasma membrane it may be preferable to replace the Potter-Elvehjem homogenizer with a loose-fitting Dounce homogenizer (clearance 0.1-0.3 mm) using about 10 strokes of the pestle and filter before processing further.
3. It is often advantageous to guard against possible protein hydrolysis in the homogenate by including a cocktail of protease inhibitors in HM: 1 mM phenylmethylsulphonyl fluoride (PMSF) and 2 µg/ml each of antipain, leupeptin and aprotinin.

5. References

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