PREPARATION OF PROTEIN SAMPLES FOR 2D GELS

1. Grind 2 g tissue in liquid N_2 and transfer to 5 ml of **homogenization buffer** in a 30 ml tube.
2. Spin 10K 10' at 4°C.
3. Filter samples through cheesecloth.
4. To supernate add 1 ml **nuclease solution**. Place on ice for 30'.
5. Add equal volume **homogenization buffer** to supernate.
6. Add 1.06 g recrystallized urea per ml solution. Warm to 37°C to dissolve.
7. Store at –70°C.

Homogenization Buffer (50 ml) (make fresh)

18% sucrose	15 ml 60%
10 mM $MgCl_2$	0.5 ml 1 M
100 mM Tris-HCl, pH 8.0	2.5 ml 2 M
40 mM β-mercaptoethanol	140 μl

Nuclease solution (10 ml)

200 mM Tris pH 7.2	1 ml 2 M
50 mM $MgCl_2$	500 μl 1 M
1 $mgml^{-1}$ RNAase A	10 mg
1 $mgml^{-1}$ DNAaseI	10 mg

Notes:
If have problems with proteases, add inhibitors to extraction buffer: 1mM PMSF, 1mM benzamidine (BAM), 5 mM ε-aminocaproic acid (ACA).

Final protein concentration should be about 0.5 $mgml^{-1}$. Load about 200 μl per tube.