Preparation of protein samples from mouse hearts

(K. Hong 01/2001)

Materials needed:

sodium pentobarbitol, 0.1cc per mouse	kimwipes
ice-cold PBS	mortar and pestle
1cc syringe with needle	liquid N_2
balance and weigh boats	labeled tubes on ice
ethanol bottle	SDS-PAGE sample buffer
stainless steel scissors, forceps, and spatula	blue pad

1. The mice are given a lethal dose of anesthetic, sodium pentobarbitol, diluted in PBS. The amount needed varies depending on the size of the animal, but I usually use 0.1cc anesthetic diluted with 0.1cc PBS per mouse. Administer the drug by injecting just under the skin of the abdomen. It usually takes about 30 seconds for the animal to go down.

2. Weigh the mouse and record body weight.

3. Lay the animal on its back. Make sure there is no response to pinched tail or foot.

4. Spray ethanol on chest fur. Pick up skin about halfway down abdomen and cut just under the skin in a V-shape up to the front legs. This cut should remove the skin but leave the peritoneal membrane intact. The entire rib cage should be visible underneath.

5. Holding the xiphoid process (the piece of cartilage at the end of the sternum), lift the rib cage up. Use the point of the scissors to cut through the peritoneal membrane and through the ribs in the same V-shape as before. One snip on each side is usually enough to cut through the diaphragm. Continue lifting and cutting back the ribs until the heart is exposed. At this point it should still be contracting.

6. With the forceps, grab the apex of the heart and lift out of the chest. The atria should be very dark red and full of blood. Snip above the atria (they will drain and shrink) to excise the heart.

7. Rinse the heart in ice-cold PBS. Blot dry on a kimwipe.

8. Weigh the heart and record heart weight.

9. Fill the mortar with liquid N_2. Briefly cool a stainless-steel spatula in the liquid N_2. Drop in the whole heart. Pulverize the heart in liquid N_2 using the mortar and pestle. After the nitrogen has evaporated, scoop out the frozen powder with the pre-cooled spatula and put into a labeled tube on ice. Spin down, and immediately add buffer. If using SDS-PAGE sample buffer, add at least 1ul/mg of tissue. Work quickly at this step so that the pulverized tissue does not thaw out.

10. The samples can now be stored at -80C until ready to use. Normally for gels I boil 2 minutes, sonicate 10 minutes, vortex, and quantitate the samples. This can be done now or right before loading the gel.

11. If desired, take another tail biopsy (1-2 cm) to obtain a DNA sample (see DNA prep protocol).

12. Wrap the carcass in a blue pad and take to 3rd floor cold room for disposal.