Perfusion fixation of tissue.
Perfusion fixation is strongly recommended for tissues (especially nervous tissue) and is discussed in the following references: Robards and Wilson, (1993) and Griffiths (1993). A sample protocol for brain fixation through the heart is given below.

Perfusion fixation of the brain through the heart. (see Thompson et al., 1992)

- The deeply anaesthetised mouse* is pinned out with its ventral surface uppermost. The ribcage is exposed and retracted.

- A 25 gauge microlance is pushed into the left ventricle through which is perfused, via a peristaltic pump, ice-cold 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1% (w/v) sodium nitrite. The right atrium is cut under slight positive pressure from the perfusate. The animal is exsanguinated in this manner for 5 minutes (~25 ml buffer).

- The perfusate is exchanged for freshly prepared, ice-cold fixative (normally 4% paraformaldehyde + 0.05% glutaraldehyde in phosphate buffered saline or other suitable buffer .

- The fixation procedure is continued for 20 minutes (100 ml) after which the brains are dissected out and immersed in fixative, at 4 ^{o}C, overnight.

- Fixed, PBS-washed brains are further processed for embedding and sectioning.

*appropriate licences are needed to perform such procedures.