

PERFUSION PROTOCOL

PREPARING FIX SOLUTION

For 100 ml of 2% formaldehyde/2.5% glutaraldehyde in 0.15M sodium cacodylate buffer:

- 1) Heat 30 ml of ddH₂O to 60°C
- 2) Add 2 g paraformaldehyde
- 3) Add 2-3 drops 1 N NaOH
- 4) When solution clears filter through a #1 Whatman filter
- 5) Add 10 ml of 25% glutaraldehyde (EM grade)
- 6) Add 50 ml 0.3M sodium cacodylate stock solution pH 7.4
- 7) Bring total volume to 100 ml with ddH₂O
- 8) Warm to 35°C for perfusion.

PREPARING RINGER'S SOLUTION

Need 250 ml to perfuse 2-4 rats.

- 1) Add the following components:
 - 2.5 ml Na₂HPO₄ (18 g/L)
 - 24.75 ml NaCl (79.8 g/L)
 - 2.5 ml KCl (37.5 g/L)
 - 2.5 ml MgCl₂ · 6 H₂O (20.0 g/L)
 - 6.25 ml NaHCO₃ (50.0 g/L)

- 2) Bring total volume to 250 ml.

Bubble 95% air/5% CO₂ through the solution @ 35°C for 5 min.

- 3) Add 2.5 ml CaCl₂ · 2 H₂O (30.0 g/L).

If high quality ultrastructure is desired, can add xylocaine (anesthetizes smooth muscle) and heparin (prevents blood clotting).

PERFUSION

Turn on pump and allow Ringer's solution to reach pump. Turn valve to allow fix solution to reach valve. Watch and make sure that air is removed from both lines. Turn valve back to Ringer's and set pump rate to proper setting depending on size of animal.

For mice, use 25 gauge needle
For medium rats, use 22 gauge needle
For large rats, use 18 gauge needle

Set out the necessary surgical instruments: large and small scissors, hemostat, tweezers, and iris scissors.

Anesthetize rats by giving an intraperitoneal injection of nembutol (0.1 cc /100gm body weight). Ensure the needle is in the peritoneal cavity by drawing back needle and checking for air (no blood).

Allow anesthetic to take effect and test rat using tail pinch and paw prick. Ensure that breathing does not stop.

Pin anesthetized rat to blue board. Using small scissors and tweezers, lift up skin below rib cage and cut until liver is visible. Cut upward along sides of body cavity until diaphragm is visible. Cut diaphragm and then quickly cut up along sides of body, being very careful not to damage heart. Once heart is sufficiently exposed, use hemostat to pull skin up over rat's head. Place needle into left ventricle and then cut right atrium with iris scissors. Turn on flow of Ringer's solution. Heart should continue to beat and liver should quickly turn pale in color. Perfuse with Ringer's for about one minute. Switch valve on pump to fix solution. The heart should stop beating and the body should begin to stiffen. Perfuse with fix solution for 3-5 minutes. Take needle out of heart. Remove tissue desired and place in ice cold fix for 1 hour.