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Mouse Heart Perfusion - Ultrastructural Analysis (Using Karnovsky's Fixative)

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We use this protocol and it's working

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Abstract

Optimal fixation for mouse tissue analysis.

Materials

1) (Cacodylic acid, sodium salt trihydrate: Ted Pella, cat # 18851)
2) (Paraformaldehyde, Prills: EMS cat. #19202)
3) 25% glutaraldehyde (EM grade). (Ted Pella, Cat. # 18426)
4) Detrose
5) Drug: Nembutal “Pentobarbital Sodium Injection, USP, peritoneal injection, minimal dose (IACUC number is 2.5mg/ 20 g mouse; 50cc stock or 1000mg/ 20ml).
6) 2% Lidocaine hydrochloride
7) 1 N NaOH
8) 95% O2/5% CO2 gas tank
9) Ringer's solution
10) NaCl (79.8 g/L stock, 4 ºC fridge)
11) Na2HPO4 (18 g/L stock, room temperature)
12) KCl (37.5 g/L stock, 4 ºC fridge)
13) MgCl2 . 6 H20 (20.0 g/L stock, 4 ºC fridge)
14) CaCl2 X 2H2O (30.0 g/L stock 4 ºC fridge)
15) NaHCO3 (50.0 g/L stock, 4 ºC)
16) 25 gauge needle
17) 3X125ml Erlenmeyer flask
18) 100ml Erlenmeyer flask
19) water bath
20) #1 Whatman filter
21) Funnel
22) Parafilm
23) Heating Plate
24) Heprin (1,000 use units per mL)
25) Perfusion pump
26) Surgical instruments: large and small scissors, hemostat, tweezers, and iris scissors.
27) Stir bar

Safety warnings

Make sure perfusion is done in fume hood. Wear PPE.

Ethics statement

Protocol Number: S06211
Nembutal “Pentobarbital Sodium Injection, USP, peritoneal injection, minimal dose (IACUC number is 2.5mg/ 20 g mouse; 50cc stock or 1000mg/ 20ml).

Preparing Karnovsky's Modified Fixative Solution

1 For 100 ml of **2.0% paraformaldehyde - 2.5% glutaraldehyde**in 0.15M sodium cacodylate buffer, pH 7.4 with 2mM $CaCl_2$:

2 Add 2 g prills paraformaldehyde into 125ml Erlenmeyer flask. **(Paraformaldehyde, Prills: EMS cat. #19202)**

3 Add 30 ml of ddH_2O to flask.

4 Add about 3-5 drops of 1 N NaOH and allow to mix with stir bar on heating plate at 60-65^{o}C until solution clears.

5 Filter cleared solution through a #1 Whatman filter (using funnel) into a 100 ml graduated cylinder.

6 Add 50 ml 0.3M cacodylate stock solution (pH 7.4) **(Cacodylic acid, sodium salt trihydrate: Ted Pella, cat # 18851)**

7 Add 10 ml of **25% glutaraldehyde (EM grade)**. **(Ted Pella, Cat. # 18426)**.

8 Add 1 mL of 0.204 M CaCl2 X 2H2O.

9 Bring total volume to 100 ml with ddH_2O and check pH is 7.4.

10 1. Pour into 100ml Erlenmeyer flask, cover with Parafilm and keep at 35^{o}C in a water bath for perfusion.

Preparing Ringer's Solution

11 For 100 ml:

Add the following components:

9.9 ml NaCl stock solution (79.8 g/L; 1.365M)
1.0 ml Na_2HPO_4 stock solution (18 g/L; 0.127M)

1.0 ml KCl stock solution (37.5 g/L; 0.503M)
1.0 ml MgCl2. 6 H_2O stock solution (20.0 g/L; 0.098M)
2.5 ml $NaHCO_3$ stock solution (50.0 g/L; 0.595M)

12 Bring total volume to 95 ml.

13 Add 0.2 grams dextrose.

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

15 After bubbling with CO2, add 1.0 ml $CaCl_2$. 2 H_2O stock solution (30.0 g/L; 0.204M).

16 Add 1.0 mL of 2% Lidocaine (anesthetizes smooth muscle) and 2.5 mL of 1000U/mL heparin (prevents blood clotting).

Perfusion

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

18 For mice, use 25 gauge needle.

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

20 Anesthetize animal by giving an intraperitoneal injection of 0.05ml “Pentobarbital Sodium Injection, USP, peritoneal injection, minimal dose (IACUC number is 2.5mg/ 20 g mouse; 50cc stock or 1000mg/ 20ml). Ensure the needle is in the peritoneal cavity by drawing back needle and checking for air (no blood).

21 Allow several minutes for anesthetic to take effect and test depth of anesthesia using tail pinch and paw prick. Ensure that breathing does not stop.

22 Secure limbs of animal. 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. Try to avoid injuring organs. Cut diaphragm and then *quickly* cut up along sides

of body, being very careful not to damage heart and trying to avoid lungs. 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. The **heart should continue to beat and liver should quickly turn pale in color.** Perfuse with Ringer's for about 1 minute at a rate of approximately 5 mL / 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 10 minutes. Take needle out of heart.

23 Dissect the brain without any artificial damage and place in vial containing same fixative and keep at 4°C for 2 hours.

Protocol references

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