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Perfusion-fixation procedure for adult rhesus monkeys

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ABSTRACT

This protocol details Perfusion-fixation procedure for adult rhesus monkeys.

ATTACHMENTS

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MATERIALS TEXT

Solutions to prepare:

- Pentobarbital ([M]25 mg/mL solution).
- **Ringer's solution**

Dissolve the following reagents, in order, in 1000 mL of distilled water.

A	B
Reagent	**Quantity**
HEPES	0.60 g
NaCl	11.86 g
KCl	0.223 g
CaCl2	0.353 g
NaHCO3	2.18 g
KH2PO4	0.177 g
MgSO4	0.32 g
D-glucose	1.8 g

- Fixative 2.5 L

1. Paraformaldehyde 96% **Fisher Scientific Catalog #AC416785000**)
2. 0.1% Glutaraldehyde **Electron Microscopy Sciences Catalog #16220**
3. [M]**0.1 Molarity (M)** phosphate buffer, pH**7.4**

Glutaraldehyde must be added to the fixative solution only at the time of

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perfusion.

FIXATIVE SOLUTION:

PREPARE THIS SOLUTION UNDER A HOOD.

- Final volume: **1 L**
- Concentration:

A	B
Paraformaldehyde	4%
Glutaraldehyde	0.1%

- Heat **500 mL** distilled water until the temperature reaches **60 °C** .
- Dissolve **40 g** paraformaldehyde with strong stirring action for **00:20:00** .
- Add NaOH drop by drop until the solution is almost clear.
- Let the solution cool for **01:00:00** .
- Filter the solution in another beaker and add **500 mL** PB (**0.2 Molarity (M)** pH **7.4**)

KEEP IN THE FRIDGE.

- Just before the perfusion, substitute **4 mL** of fixative by **4 mL** of glutaraldehyde 25%.

ANTI-FREEZE SOLUTION:

Prepare the following solution in the same order:

For **1000 mL** solution:

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A	B
Sodium phosphate monobasic, NaH2PO4·H2O	13.8 g
Sodium phosphate dibasic heptahydrate, Na2HPO4·7H2O	25.8 g
Distilled water	400 ml
Ethylene glycol	300 ml
Glycerol	300 ml

Sections can be stored in this solution at **-20 °C** freezer for many months without loss of ultrastructural preservation and tissue antigenicity.

Equipment and room needed:

- Necropsy room equipped with proper exhaust and ventilation to reduce exposure to aldehydes.

General-Purpose Peristaltic Pumps
Peristaltic Pump
Fisherbrand™ GP1000 13310656

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- Oxygen tank (95% O_2, 5%CO_2).
- Surgical instruments (scalpels, hemostats of various sizes, bone cutter, scissors, bone saw, forceps, rongeur).

BEFORE STARTING

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BEFORE STARTING

Wear PPE before entering the perfusion room, i.e., gloves, coat, hair net, safety glasses, face mask and face shield or goggles.

Make sure the Hazardous Waste container is not full. Replace if full.

Perfusion-fixation procedure for adult rhesus monkeys 1d

1 

Add glutaraldehyde to the fixative solution.

2 Start oxygenation of the Ringer's solution.

3 Anesthetize the animal with Ketamine ([M]**10 mg/kg** , i.m.) or Telazol ([M]**3 mg/kg** - [M]**5 mg/kg** , i.m.) in its home cage and bring to the necropsy room.

4 Place an i.v. catheter.

5 Perform a tracheal intubation.

6 Inject **1 mL** of heparin (i.v.).

7 Inject an overdose of pentobarbital ([M]**25 mg/kg** i.v.).

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8 Ensure the absence of all reflexes, including toe pinch reflexes and brainstem reflexes, such as corneal reflexes.

9 Cut the thoracic cage to provide access to the heart, clamp the descending aorta, open the pericardium and expose the heart muscle, make an incision of the right atrium and insert the perfusion needle in the left ventricle.

10 Artificially ventilate the animal through the tracheal tube during the Ringer's solution perfusion.

11 

Infuse transcardially ~300- **400 mL** of cold oxygenated Ringer's solution at a rate of 80-90 ml/min through a needle (14G; 1.5 inch long) inserted in the left ventricle. Let the infused solution exit the vascular system through a hole in the right atrium.

12 

Perfuse **2.5 L** of fixative, starting at a rate of 80-90 ml/min for the first liter and then reduce the rate to 50 ml/min for the remaining solution.

13 After perfusion, fix the animal's head in a stereotaxic frame and open the skull using a bone saw and rongeur.

14 Cut the brain in **10 mm** -thick blocks in the coronal stereotaxic plane, remove the resulting blocks of tissue from the skull and post-fix them for **24:00:00** in 4% paraformaldehyde solution in PB (**0.1 Molarity (M)**, pH **7.4**) at **40 °C**. 1d

15 Transfer the tissue into a phosphate-buffered saline (PBS, **0.01 Molarity (M)**, pH **7.4**) solution.

16 Cut the brain in **50 µm** -thick sections using a vibrating microtome or a freezing microtome

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and store in a -20 °C freezer in an anti-freeze solution (**Please refer material section**) until further processing.

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