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Preparation of 4% paraformaldehyde solution for transcardial perfusions and histology

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We use this protocol and it's working



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Abstract

This protocol describes the safe preparation of 4% paraformaldehyde solution for use in tissue fixation in preparation for histology and immunohistochemistry. Paraformaldehyde is extremely toxic and must be handled with great care. We therefore also provide advice for the safe handling and disposal of this solution.

Attachments



[it72bj7ap.docx](#)

95KB

Materials

Equipment:

- Weighing spatulas
-  2 L heat resistant (up to 500 degrees) glass beaker
- Hot plate with stirrer
- Magnetic stirrer bar
- Ceramic vacuum filtration system with conical flask
-  2 L Schott bottle

Consumables:

- Whatman Filter Paper
- Formaldehyde spills kit

Key reagents:

-  Paraformaldehyde (prilled 95%) Merck MilliporeSigma (Sigma-Aldrich) Catalog #441244
- Sodium hydroxide pellets (Thermo #CPC-AJA482)
- Hydrochloric acid

Solutions:

- **Buffer A:** [M] 0.2 Mass Percent NaH_2PO_4
 1.  13.8 g in  500 mL dH₂O
- **Buffer B:** [M] 0.2 Mass Percent Na_2HPO_4
 1.  14.194 g in  500 mL dH₂O

Safety warnings

1. This procedure will take place entirely inside the fume hood.
2. Place designated PFA scale and hot plate and stirrer inside the fume hood, these should not be used for any other purpose.
3. Adequate PPE including double nitrile gloves, lab gown, safety glasses should be worn at all times, with gloves replaced regularly and breaks taken at least every 15 minutes throughout.
4. Place PFA in progress sign on exterior of room door, close the door.



Solution preparation

- 1 Weigh  40 g PFA.
- 2 Add to  500 mL H₂O in beaker with flea. 
- 3 Heat solution to  60 °C -  65 °C on the heat plate with stirrer (use lowest possible heat setting).
- 4 Add 1-2 pellets of NaOH to dissolve PFA powder. 
- 5 Allow solution to cool to  Room temperature .
- 6 Filter dissolved PFA using ceramic vacuum filtration system into a conical flask.
- 7 Mix PFA solution with  120 mL Buffer A and  380 mL Buffer B (recipes below).
- 8 Adjust pH solution to 7.4 using 2N HCl. Only use the pH meter designated for PFA preparation.
- 9 Transfer solution into 1 L Schott bottle. 
- 10 Label solution according to GHS guidelines.
- 11 Store at  4 °C .
- 12 Wash hands with soap and water once finished and before leaving the laboratory. 

Spills

13 The following general procedures can be used for spills involving **small volumes** (mL size) of dilute solutions of formaldehyde (e.g.  100 mL fixative solution of 4% formaldehyde) contained within a fume hood:

13.1 Wear appropriate personal protective equipment(PPE).

Note

This should include a reusable respirator if there is not sufficient local area ventilation.

13.2 Remove any ignition source from the spill area (particularly if the solution has been stabilised with methanol).

13.3 If possible, boost ventilation to the area.

13.4 Absorb any residual solution with absorbent.

13.5 Clean spill area with detergent and water.

13.6 Dry decontaminated spill area with paper towels.

13.7 Dispose of any absorbent material along with other chemical waste through the university hazardous waste program.

14 **If a large volume** of concentrated formaldehyde solution is spilled (e.g>  1 L , 37% formaldehyde) or if the spill occurs outside a chemical fume hood or ventilated area, immediately:

14.1 Alert others working in the area.

14.2 Evacuate the area.



14.3 Restrict the access to the area.

14.4 Contact Laboratory Supervisor for use of formaldehyde spills kit containing a urea-based formaldehyde neutralizer (e.g. Spill X-FPTM) which will chemically react with formaldehyde to form a polymer product that is no longer volatile and much less hazardous. These formaldehyde spill neutralizers are commercially available. Locate the spills kit within easy access to where formaldehyde is handled or stored.

Exposure

15m

15 Formaldehyde is very water soluble and will therefore affect mucous membranes.

Note

Symptoms of acute exposure can result in eye and throat irritation, coughing, wheezing, chest tightness, bronchitis, laryngitis, clouding of the cornea and loss of vision.

16 The following first aid procedure should be promptly undertaken;

16.1 Exposure via inhalation.

- Immediately move the person to fresh air.
- Contact a first aider and/or refer the person to the University medical center.
- If the person is having respiratory difficulties, call an ambulance.

16.2 Skin or eye exposure:

- Remove contaminated clothing / contact lenses.
- Immediately flush with tepid water for at least  00:15:00 .
- Contact a first aider for assistance.
- If there is severe irritation, refer to the University medical centre.

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