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Fixation of human kidney biopsies for ultrastructural and molecular preservation for clinical research

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We use this protocol and it's working

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Disclaimer

Abstract

This protocol details the fixation of 1 mm diameter human kidney needle core biopsies in the clinic, compatible with ultrastructural and molecular preservation for downstream clinical research applications, including those that use high resolution imaging with light, X-ray and electron microscopes.

Guidelines

Health & Safety requirements:
Ensure you are familiar with all relevant local Health and Safety documents and procedures (Material Safety Data Sheets, Procedural Risk Assessments, Standard Operating Procedures etc.) before starting.

Procedural Risk Assessments: Handling of formaldehyde fixative

Abbreviations:
- PB: Phosphate buffer
- FA: Formaldehyde

Materials

Materials:

1. Fridge, 4ºC
2. Balance and weighing dishes
3. pH meter
4. Serological pipette controller
5. 25 ml serological pipettes (sterile)
6. 5 ml serological pipettes (sterile)
7. Magnetic stirrer plate
8. Magnetic stirring bars (for stirring buffers)
9. Measuring cylinders (for PB buffer prep)
10. 500 mL glass beakers (for PB buffer prep)
11. Nalgene rapid-flow sterile disposable filter units with nylon membrane, 500 mL, 0.2 µm pore **Thermo Fisher Catalog** #151-4020
12. 5 ml amber screw cap centrifuge tubes (sterile) **Eppendorf Catalog** #0030122330
13. 30 mL polypropylene Universal Containers (sterile) **Sterilin Thermo Fisher Catalog** #128CP
14. Parafilm **Agar Scientific Catalog** #AGG3398

Chemicals:

1. ddH_2O; double distilled water (Sterile). Deionised ultra-pure water can be used in place of ddH_2O.
2. Na_2HPO_4; di-Sodium hydrogen orthophosphate, anhydrous **VWR (Avantor)Catalog** #102494C
   OR
   Na_2HPO_4•$2H_2O$; di-Sodium hydrogen orthophosphate dihydrate **VWR (Avantor)Catalog** #28029.260
3. NaH_2PO_4•H_2O; Sodium dihydrogen orthophosphate monohydrate **VWR (Avantor)Catalog** #102454R
   OR
   NaH_2PO_4•$2H_2O$; Sodium dihydrogen orthophosphate dihydrate **VWR (Avantor)Catalog** #28015.261
4. Formaldehyde 36% (aq) stock **TAAB Catalog** #F003

Formaldehyde	**State:** Liquid	**Hazard Codes:** H226, H301, H311, H314, H317, H330, H335, H341, H350, H370	

Safety warnings

Safety critical: All procedures designated safety critical must be performed in a fume hood and the following personal protective equipment (PPE) must be worn - lab coat, gloves, and eye protection.

Ethics statement

Prior ethics approval should be obtained before working with human tissue according to local rules.

Procedure: Immersion fixation of human tissue

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Note

- Human biopsies are usually fixed in formalin (that may contain methanol) and embedded in paraffin, or snap frozen. Both processes destroy the ultrastructure of the sample and are unsuitable for preserving samples that will later be imaged by high resolution light, X-ray or electron microscopy for clinical research.
- In some cases, human biopsies are fixed using glutaraldehyde, or a mix of (para)formaldehyde and glutaraldehyde, for diagnostic electron microscopy. Glutaraldehyde can destroy or mask antigens, leading to issues with downstream immunolabelling for clinical research.
- This protocol was developed to preserve both ultrastructure and molecular antigenicity of human biopsies for downstream clinical research using high resolution imaging modalities.
- The protocol was developed for 1 mm diameter human kidney needle core biopsies. Appropriate solution volumes are indicated.
- For larger human biopsies/tissue, it is recommended that the sample is dissected so that it is thinner than 1 mm in at least one dimension to allow good penetration of the fixative.
- Samples must not dry out during fixation and storage, as this will destroy the ultrastructure.

Prepare buffer and fixative aliquots

2 Prepare Phosphate Buffer (PB) aliquots for transfer to the clinic

2.1 Prepare PB Solution X by dissolving
EITHER
14.91 g of Na_2HPO_4 (anhydrous) in 525 mL of ddH_2O
OR
18.69 g of $Na_2HPO_4 \cdot 2H_2O$ (dihydrate) in 525 mL of ddH_2O
Filter sterilise and store at 4 °C

2.2 Prepare PB Solution Y by dissolving
EITHER
13.80 g of $NaH_2PO_4 \cdot H_2O$ (monohydrate) in 500 mL ddH_2O
OR
15.60 g of $NaH_2PO_4 \cdot 2H_2O$ (dihydrate) in 500 mL ddH_2O

Filter sterilise and store at 4 °C

2.3 Prepare [M] 0.2 Mass Percent PB pH 7.4

1. Mix 405 mL of PB Solution X with 95 mL of PB Solution Y
2. Adjust the pH to pH 7.4 using hydrochloric acid (HCl)
3. Filter sterilise and store at 4 °C

2.4 Prepare 25 x 22.2 mL aliquots of [M] 0.113 Molarity (M) PB pH 7.4

1. Mix 450 mL of filter sterilised [M] 0.2 Molarity (M) PB pH 7.4 with 349.2 mL of ddH2O in sterile glassware
2. Using a sterile 25 mL serological pipette, add 22.2 mL of [M] 0.113 Molarity (M) PB to each 30 mL polypropylene universal tube
3. Label each tube with contents and date and store at 4 °C

3 Prepare 25 x 2.8 mL aliquots of 36% EM-grade formaldehyde (FA) **Safety Critical**

3.1 Using a sterile 5 mL serological pipette, add 2.8 mL of 36% FA to each 5 mL amber centrifuge tube **Safety Critical**

3.2 Label each tube, place in a labelled box which clearly displays the hazard warning symbols, and store at Room temperature **Safety Critical**

4 Transfer buffer and fixative aliquots to the clinic

Collection of biopsies in EM-grade fixative

5 Prepare a vial of EM-grade fixative for each biopsy on the day of sample collection **Safety Critical**

> Note
>
> Normally this should be done in a fume hood. In a clinical setting, a fume hood may not be available. If this is the case, follow local rules, and take care to open the FA vial for the minimal time required to dispense it into the PB vial.

5.1 For each biopsy, add one FA aliquot to one PB aliquot **Safety Critical**
This will make a vial of [M] 4 % (v/v) FA in [M] 0.1 Molarity (M) PB

5.2 Prior to tissue collection, ensure that the appropriate labelled vial containing primary fixative is at hand so that time between tissue collection and fixation is minimised.

5.3 Immediately after collection, add the biopsy to the fixative vial. The fixative vial should only be opened for the time needed to allow insertion of the sample. PPE should be worn **Safety Critical**

Note

If the sample cannot be immediately immersed in fixative at the bedside or in theatre, then it should be immersed as soon as safe and practical to do so, for example, on arrival in the pathology lab. However, it is critical to note that any drying of the tissue will result in deterioration of the ultrastructure.

5.4 Fix biopsy for at least 12:00:00 at Room temperature 12h

Note

We suggest that the biopsy is fixed for 12 h at room temperature to help ensure that any unknown pathogens in the sample are inactivated. The biopsy should then be stored in the fridge at 4ºC until further processing.

Long term storage of fixed biopsies

6 Transfer samples to a vial containing at least 1 mL of [M] 1 % (v/v) FA in [M] 0.1 Molarity (M) PB and store at 4 °C .

Note

We suggest that the storage fixative be replaced with freshly made 1% FA in 0.1 M PB every 2 to 3 months. Formaldehyde is known to undergo slow reactions including oxidisation to formic acid. Polymerisation may also occur with associated reduction in the availability of formaldehyde monomers. The temporal kinetics of these reactions in a buffered system of 1% FA at 4ºC appears to be unknown.