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Karnovsky's Modified Fixative

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We use this protocol and it's working

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Abstract

Fixative for mice and rat tissues to be used during perfusion and immersion fixation.

Guidelines

Preparing Fix solution (2% paraformaldehyde and 2.5% glutaraldehyde)

For 100 mL of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.15M cacodylate buffer solution at 7.4 pH.

Materials

- 100mL Erlenmeyer flask
- 100mL graduated cylinder
- #1 Whatman filter
- Paraformaldehyde PRILLS (Catalog no. 19202)
- Glutaraldehyde EMS (Catalog no. 16220)
- Sodium Cacodylate buffer TED PELLA, INC. (Catalog no. 18851)
- Stir bar

Safety warnings

! Always wear appropriate PPE.

Before start

Make sure heating plate for dissolving paraformaldehyde is between 60-70° C.

- 1 Add 2.0 grams paraformaldehyde to a 100mL Erlenmeyer flask.
- 2 Add 35mL of DDH₂O to 100mL to a Erlenmeyer flask.
- 3 Heat paraformaldehyde in DDH₂O to 60-70°C. Use stir bar to mix.
- 4 Add 2-3 drops 1N NaOH.
- 5 Filter solution with #1 Whatman filter into a 100mL graduated cylinder.
- 6 Add 10.0 ml of 25% glutaraldehyde.
- 7 Dilute with DDH₂O to a total volume 49.0 ml.
- 8 Add 1.0 ml of 0.204 M CaCl₂·2H₂O.
- 9 Add 50 ml 0.3M cacodylate buffer stock solution at pH 7.4.
- 10 Volume should be 100 ml. Transfer fixative into a 100mL Erlenmeyer flask.
- 11 Keep in bath water 35°C.