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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

Troubleshooting

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 DDH_2O to 100mL to a Erlenmeyer flask.

3 Heat paraformaldehyde in DDH_2O to 60-70ºC. 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 $CaCl2\text{-}2H_2O$.

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 35ºC.