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Preparation of fresh paraformaldehyde for mouse perfusion and tissue fixation

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Protocol status: Working

We use this protocol and it's working



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Abstract

Hnasko lab - preparation of fresh PFA for ms perfusion and tissue fixation

Materials

paraformaldehyde (PFA, EMS, cat# 19210)

10X PBS (Fisher bioreagents, cat# BP399-1)

vacuum filtration flask (ThermoScientific, cat# 567-0020).

Safety warnings

 **Caution:** Paraformaldehyde is toxic. Please read the MSDS before working with this chemical. Gloves, mask and safety glasses should be worn and solutions made inside a fume hood.



1. For 1 L of 4% paraformaldehyde, warm up 700 mL of ddH₂O in a glass beaker using microwave until 40-60 °C .
2. Place on heated stir plate in a chemical fume hood, add a magnetic stir bar and start stirring and insert thermometer into solution.
3. Weigh 40 g of granular paraformaldehyde (PFA, EMS, cat# 19210).
4. Add PFA to the heated ddH₂O solution and place on a stir plate in a chemical fume hood. Insert thermometer. Stir and heat to approximately 60 °C (paraformaldehyde flash point = 71°C). Avoid overheating.
5. The powder will not immediately dissolve into solution. Slowly raise the pH by adding about 1 mL NaOH 10N until the solution clears.
6. Once the paraformaldehyde is dissolved, add 100 mL 10X PBS (Fisher bioreagents, cat# BP399-1).
7. Cool solution to Room temperature before adjusting pH, can place on ice to speed cooling.
8. Adjust pH to ~ 7.4 with small amounts of 12N HCl.
9. Adjust the volume of the solution to 1 L with ddH₂O.
10. Filter the solution using a vacuum filtration flask (ThermoScientific, cat# 567-0020). Label and store in sealed container at 4 °C .