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**Protocol status:** Working  
 We use this protocol and it's working

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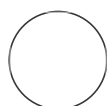
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## Kordower Lab Solution Recipes

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### ABSTRACT

Kordower Lab Solution Recipes

### ATTACHMENTS

[Kordower Lab Solutions.docx](#)

### MATERIALS

Gelatin Type A  
 Chromium Potassium Sulfate  
 Sucrose  
 Ethylene Glycol  
 Sodium phosphate monobasic (NaH<sub>2</sub>PO<sub>4</sub>)  
 Sodium phosphate dibasic (Na<sub>2</sub>HPO<sub>4</sub>)  
 NaCl  
 Trizma base  
 Trizma hydrochloride  
 Triton X-100  
 Paraformaldehyde  
 [M] 10 Molarity (m) NaOH  
 Sodium phosphate monobasic monohydrate (NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O)  
 Sodium phosphate dibasic heptahydrate (Na<sub>2</sub>HPO<sub>4</sub> · 7H<sub>2</sub>O)  
 Picric Acid Solution

**Keywords:** solutions

## Slide Subbing Solution

- 1 Warm 500 mL of dH<sub>2</sub>O to 50 °C to 60 °C, once at temperature, turn off heat, add and mix until dissolved:
  - 1.25 g Gelatin Type A
  - 0.125 g Chromium Potassium Sulfate

## Cryoprotectant Solution

- 2 In a 4000 mL beaker, begin with 2000 mL of 0.2 Molarity (m) Phosphate-Buffered Saline (PBS), pH 7.2  
(or 2000 mL dH<sub>2</sub>O + 2.8 g NaH<sub>2</sub>PO<sub>4</sub> + 10.9 g Na<sub>2</sub>HPO<sub>4</sub> + 18.5 g NaCl, pH 7.2)  
Add and mix until dissolved:
  - 1200 g Sucrose
  - 1200 mL Ethylene GlycolBring to 4000 mL final volume with additional PBS

## Phosphate-Buffered Saline (PBS)

- 3 Make 1000 mL 7X concentrate and mix until dissolved:
  - 1000 mL dH<sub>2</sub>O
  - 9.72 g Sodium phosphate monobasic (NaH<sub>2</sub>PO<sub>4</sub>)
  - 38.3 g Sodium phosphate dibasic (Na<sub>2</sub>HPO<sub>4</sub>)
  - 64.8 g NaClAdd above concentrate to 6000 mL dH<sub>2</sub>O to make 7000 mL of 1X solution. Adjust pH 7.2, if needed.

## Tris-Buffered Saline (TBS)

- 4 Make 1000 mL 7X concentrate and mix until dissolved:
  - 1000 mL dH<sub>2</sub>O
  - 6.8 g Trizma base
  - 46.3 g Trizma hydrochloride
  - 61.4 g NaCl

Add above concentrate to 6000 mL dH<sub>2</sub>O to make 7000 mL of 1X solution. Adjust pH 7.4, if needed.

## Dilution Media (DM)

5 Make 1000 mL 7X concentrate and mix until dissolved:

- 1000 mL dH<sub>2</sub>O
- 6.8 g Trizma base
- 46.3 g Trizma hydrochloride
- 61.4 g NaCl
- 3.5 mL Triton X-100

Add above concentrate to 6000 mL dH<sub>2</sub>O to make 7000 mL of 1X solution. Adjust pH 7.4, if needed.

## 4% Paraformaldehyde Solution (PFA)

17m

6 Solution A (make in 4000 mL beaker in fume hood)

17m

- 160 g paraformaldehyde (weigh in fume hood)
- Heat 2000 mL dH<sub>2</sub>O in microwave for 00:07:00 to 00:10:00 until approximately 65 °C
- Add heated dH<sub>2</sub>O to paraformaldehyde in beaker until filled to 2000 mL
- Stir on heated stir plate, maintaining 60 °C to 65 °C (do not exceed 70 °C !!)
- Add 10 Molarity (m) NaOH until solution clears (typically 10 mL to 20 mL)
- Allow solution to cool to < 30 °C, can place in ice water bath to speed cooling

7 Solution B (make in 4000 mL beaker)

- 2000 mL dH<sub>2</sub>O
- 13.6 g Sodium phosphate monobasic monohydrate (NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O)
- 80.9 g Sodium phosphate dibasic heptahydrate (Na<sub>2</sub>HPO<sub>4</sub> · 7H<sub>2</sub>O)

8 Vacuum filter Solution A into Solution B in fume hood using Büchner funnel and flask and 15 cm round filter paper

Adjust to pH 7.4, if needed

Store at 4 °C for 1-2 weeks (ok to use if pH remains the same)

## Zamboni's Fixative

17m

9 Solution A (make in 4000 mL beaker in fume hood)

17m

1. 160 g paraformaldehyde (weigh in fume hood)
2. Heat 2000 mL dH<sub>2</sub>O in microwave for 00:07:00 to 00:10:00 until approximately 65 °C
3. Add heated dH<sub>2</sub>O to paraformaldehyde in beaker until filled to 2000 mL
4. Stir on heated stir plate, maintaining 60 °C to 65 °C (do not exceed 70 °C !!)
5. Add 10 Molarity (m) NaOH until solution clears (typically 10 mL to 20 mL)
6. Allow solution to cool to < 30 °C, can place in ice water bath to speed cooling

## 10 Solution B (make in 4000 mL beaker)

- 1700 mL dH<sub>2</sub>O
- 6.6 g Sodium phosphate monobasic monohydrate (NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O)
- 94.4 g Sodium phosphate dibasic heptahydrate (Na<sub>2</sub>HPO<sub>4</sub> · 7H<sub>2</sub>O)
- 300 mL Picric Acid Solution

## 11 Vacuum filter Solution A into Solution B in fume hood using Büchner funnel and flask and 15 cm round filter paper

Adjust to pH 7.4, if needed

Store at Room temperature for up to 1 year