Kordower Lab Solution Recipes

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

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ABSTRACT

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ATTACHMENTS

Kordower Lab Solutions.docx

MATERIALS

Gelatin Type A
Chromium Potassium Sulfate
Sucrose
Ethylene Glycol
Sodium phosphate monobasic (NaH2PO4)
Sodium phosphate dibasic (Na2HPO4)
NaCl
Trizma base
Trizma hydrochloride
Triton X-100
Paraformaldehyde
[M] 10 Molarity (m) NaOH
Sodium phosphate monobasic monohydrate (NaH2PO4 · H2O)
Sodium phosphate dibasic heptahydrate (Na2HPO4 · 7H2O)
Picric Acid Solution

PROTOCOL integer ID:
89630

Keywords: solutions

Slide Subbing Solution

1 Warm 500 mL of dH_2O 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 [M] 0.2 Molarity (m) Phosphate-Buffered Saline (PBS), pH 7.2
(or 2000 mL dH2O + 2.8 g NaH2PO4 + 10.9 g Na2HPO4 + 18.5 g NaCl, pH 7.2)
Add and mix until disolved:

- 1200 g Sucrose
- 1200 mL Ethylene Glycol

Bring 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_2O
- 9.72 g Sodium phosphate monobasic (NaH2PO4)
- 38.3 g Sodium phosphate dibasic (Na2HPO4)
- 64.8 g NaCl

Add above concentrate to 6000 mL dH_2O 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_2O
- 6.8 g Trizma base
- 46.3 g Trizma hydrochloride
- 61.4 g NaCl

Add above concentrate to 6000 mL dH_2O 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_2O
- 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_2O 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

1. 160 g paraformaldehyde (weigh in fume hood)
2. Heat 2000 mL dH_2O in microwave for 00:07:00 to 00:10:00 until approximately 65 °C
3. Add heated dH_2O 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 [M] 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

7 Solution B (make in 4000 mL beaker)

- 2000 mL dH2O
- 13.6 g Sodium phosphate monobasic monohydrate (NaH2PO4 · H2O)
- 80.9 g Sodium phosphate dibasic heptahydrate (Na2HPO4 · 7H2O)

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_2O in microwave for 00:07:00 to 00:10:00 until approximately 65 °C
3. Add heated dH_2O 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 [M] 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 dH2O
- 6.6 g Sodium phosphate monobasic monohydrate (NaH2PO4 · H2O)
- 94.4 g Sodium phosphate dibasic heptahydrate (Na2HPO4 · 7H2O)
- 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