

Oct 19, 2023

Kordower Lab Solution Recipes

DOI

dx.doi.org/10.17504/protocols.io.261gedxjv47/v1

Yaping Chu¹, Scott Muller¹, Jeffrey Kordower¹

¹Arizona State University

Team Kordower



Scott Muller

Arizona State University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.261gedxjv47/v1>

Protocol Citation: Yaping Chu, Scott Muller, Jeffrey Kordower 2023. Kordower Lab Solution Recipes. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.261gedxjv47/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: October 19, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 89630

Keywords: solutions, ASAPCRN, kordower lab solution recipes kordower lab solution recipe, lab

Abstract

Kordower Lab Solution Recipes

Attachments



Kordower Lab Solutio...

18KB

Materials

Gelatin Type A

Chromium Potassium Sulfate

Sucrose

Ethylene Glycol

Sodium phosphate monobasic (NaH_2PO_4)

Sodium phosphate dibasic (Na_2HPO_4)

NaCl

Trizma base

Trizma hydrochloride

Triton X-100

Paraformaldehyde

[M] 10 Molarity (M) NaOH

Sodium phosphate monobasic monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)

Sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$)

Picric Acid Solution

Troubleshooting



Slide Subbing Solution

- 1 Warm 500 mL of dH₂O 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 dH₂O + 2.8 g NaH₂PO₄ + 10.9 g Na₂HPO₄ + 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 dH₂O
 - 9.72 g Sodium phosphate monobasic (NaH₂PO₄)
 - 38.3 g Sodium phosphate dibasic (Na₂HPO₄)
 - 64.8 g NaClAdd above concentrate to 6000 mL dH₂O 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 dH₂O
 - 6.8 g Trizma base
 - 46.3 g Trizma hydrochloride



- 61.4 g NaCl

Add above concentrate to 6000 mL dH₂O 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 dH₂O
- 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 dH₂O 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 dH₂O in microwave for 00:07:00 to 00:10:00 until approximately 65 °C
3. Add heated dH₂O 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

- 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 $\text{pH } 7.4$, if needed
Store at $4\text{ }^{\circ}\text{C}$ 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)
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 $65\text{ }^{\circ}\text{C}$
 3. Add heated dH_2O to paraformaldehyde in beaker until filled to 2000 mL
 4. Stir on heated stir plate, maintaining $60\text{ }^{\circ}\text{C}$ to $65\text{ }^{\circ}\text{C}$ (do not exceed $70\text{ }^{\circ}\text{C}$!!)
 5. Add 10 M NaOH until solution clears (typically 10 mL to 20 mL)
 6. Allow solution to cool to $< 30\text{ }^{\circ}\text{C}$, can place in ice water bath to speed cooling
- 10 Solution B (make in 4000 mL beaker)
- 1700 mL dH_2O
 - 6.6 g Sodium phosphate monobasic monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)
 - 94.4 g Sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$)
 - 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 $\text{pH } 7.4$, if needed
Store at Room temperature for up to 1 year