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Fly Functional Connectome Recipe – Extracellular Saline

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Protocol status: Working

We use this protocol and it's working

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Abstract

Saline used to dissect and image ex vivo Drosophila Brains

Materials

****Stock A****

- A1 NaCl: 60.1932 g
- A2 KCl: 2.2365 g
- A3 TES: 11.4625 g
- A4 NaHCO₃: 21.8426 g
- A5 NaH₂PO₄: 1.1998 g (Make separately to avoid precipitation)

****Stock B****

- B1 trehalose 2 H₂O: 30.2664 g
- B2 glucose (20% in H₂O): 90.08 mL
- B2 glucose: 18.016 g
- B3 CaCl₂ 2 H₂O: 2.9402 g
- B4 MgCl₂ 6 H₂O: 8.132 g

Alternative to using solutions

- CaCl₂ Solution (1M): 20 mL
- MgCl₂ Solution (1M): 40 mL

****Preparing 1X Extracellular Saline from 10X Stocks****

1. Add 3c800mL Milli-Q water to a clean 1L graduated cylinder.
2. Using a disposable 100 mL pipette add 100 mL of Stock A.
3. Mix A using a pipette - Failure to mix will cause precipitation when adding B.
4. Using a 100 mL pipette add 100 mL of Stock B.
5. Fill the graduated cylinder with Milli-Q water to 1L total.
6. Mix with Pipette
7. Store the finished 1X Extracellular Saline solution in a refrigerator.

****Reagents and Supplies****

- A1 NaCl: Sigma Aldrich, 71376-1KG
- A2 KCl: Sigma Aldrich, 60128-250G-F
- A3 TES: Sigma Aldrich, T1375-25G, [Link](<https://www.sigmaaldrich.com/US/en/product/sigma/t1375>)
- A4 NaHCO₃: Sigma Aldrich, S6297-250G
- A5 NaH₂PO₄: Sigma Aldrich, S0751-100G
- B1 trehalose 2 H₂O: Sigma Aldrich, T0167-100G, [Link](<https://www.sigmaaldrich.com/US/en/product/sigma/t0167>)
- B2 glucose: Sigma Aldrich, G7528-250G
- B3 CaCl₂ 2 H₂O: Sigma Aldrich, 21115-100ML
- B4 MgCl₂ 6 H₂O: Sigma Aldrich, 63069-100ML, [Link](<https://www.sigmaaldrich.com/US/en/product/sial/63069?srslid=AfmBOophZ3KiILX1Z5TI8echHIY0e1YvaWdEXniLSfLVldf2JisiZ38G>)
- Vacuum filter: Corning 431205, [Link](<https://ecatalog.corning.com/life-sciences/b2c/US/en/Cell-Culture/Filtration/Vacuum-Filters/Vacuum-Filtration-Systems/p/431205>)



Safety warnings

! Mix A using a pipette - Failure to mix will cause precipitation when adding B.



Recipe for 10X Stocks

- 1 Add ~500mL Milli-Q water to a clean 1L graduated cylinder.
- 2 Add ingredients for Stock A to the graduated cylinder.
- 3 Fill the graduated cylinder with Milli-Q water to 1L total.
- 4 Mix solution using a pipette.
- 5 Vacuum filter via corning vacuum membrane 0.22 micron.
- 6 Repeat steps 1 – 5 with Stock B.

Preparing 1X Extracellular Saline from 10X Stocks

- 7 Add 3c800mL Milli-Q water to a clean 1L graduated cylinder.
- 8 Using a disposable 100 mL pipette add 100 mL of Stock A.
- 9 Mix A using a pipette - Failure to mix will cause precipitation when adding B.
- 10 Using a 100 mL pipette add 100 mL of Stock B.
- 11 Fill the graduated cylinder with Milli-Q water to 1L total.
- 12 Mix with Pipette.



- 13 Store the finished 1X Extracellular Saline solution in a refrigerator.

Osmolarity

- 14 Expected osmolarity is 290 mmol / kg.

Acknowledgements

Protocol was developed from a recipe from the Vivek Jayaraman Group.