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Making Drosophila Artificial Hemolymph

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We use this protocol and it's working

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Abstract

This is a protocol for making artificial hemolymph (AHL) for Drosophila brain dissection and imaging.

Materials

NaCl
KCl
$CaCl_2$
$MgCl_2$
NaH_2PO_4
Sucrose
Trehalose
HEPES
ddH_2O
pH meter

Safety warnings

N/A

Ethics statement

N/A

Before start

Make sure to make the stock solutions ahead of making the final AHL buffer.

Making Drosophila Artificial Hemolymph (AHL)

1 Make stock solutions

1.1 **Stock Solutions**

3M NaCl	MW: 58.44	87.66g to 500mL
1M KCl	MW: 74.56	37.28g to 500mL
500mM $CaCl_2$	MW: 147.00	14.7g to 200mL
500mM $MgCl_2$	MW: 203.3	20.33g to 200mL
500mM NaH_2PO_4	MW: 155.99	15.59g to 200mL
*200mM Sucrose	MW: 342.30	34.23g to 500mL
*100mM Trehalose	MW 378.33	3.78g to 100mL
*250mM HEPES	MW: 238.31	11.92 g to 200mL

*Store at 4°

2 Make 100 mL of AHL from the stock solutions

2.1

108mM NaCl	3.6mL of 3M NaCl
5mM KCl	.5mL of 1M KCl
2mM $CaCl_2$	.4mL of 500mM $CaCl_2$
8.2mM $MgCl_2$	1.64mL of 500mM $MgCl_2$
1mM NaH_2PO_4	.2ml of 500mM NaH_2PO_4
10mM Sucrose	5mL of 200mM Sucrose
5mM Trehalose	5mL of 100mM Trehalose
5mM HEPES	2mL of 250mM HEPES
4mM $NaHCO_3$	.4mL of 1M $NaHCO_3$

**add $NaHCO_3$ last to avoid formation of precipitates

2.2 Add ddH_2O to a final volume of 100 mL

3 Adjust final pH to 7.5

4 Check osmolarity of the solution to ensure that it is at ~265 mOsm

Protocol references

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