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Version 2

🌐 Preparing 10 L of M9 buffer for nematode culture V.2

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Gurdon Institute media kitchen¹

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Protocol status: Working

We use this protocol and it's working

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Abstract


Prepare 10 litres of M9 solution for collecting and washing *C. elegans*.


Materials


MATERIALS


 double distilled water (ddH₂O)

 Di-Sodium Hydrogen Orthophosphate Dihydrate Certified AR for Analysis Fisher Chemical **Catalog #S/4450/53**

 Sodium chloride meets analytical specification of Ph.Eur Fisher Chemical **Fisher Scientific Catalog #S/3160/65**


 Magnesium Sulfate Heptahydrate Certified AR for Analysis Fisher Chemical **Fisher Scientific Catalog #M/1050/53**

 Potassium Dihydrogen Orthophosphate Certified AR for Analysis Fisher Chemical **Fisher Scientific Catalog # P/4800/53**

 SYCHEM autoclave **Syschem**

Troubleshooting

Safety warnings

 This protocol makes use of an autoclave. Make sure you know how to use it properly before starting the protocol.



1

20m

Ingredients	Quantity	
Na ₂ HPO ₄		60g
KH ₂ PO ₄		30g
NaCl		50g
Double distilled H ₂ O	up to 10L	
Add 1M MgSO ₄ after autoclaving @ 100ul/100ml		



M9_buffer_10L.xls



M9_buffer_2L.xls

2

15m

1	Dissolve ingredients in 8L double distilled H ₂ O
2	Adjust volume to 10L using double distilled H ₂ O
3	Measure or use pump to dispense aliquots accurately.
4	Label, date and autoclave.
5	After autoclaving, add 100ul 1M MgSO ₄ per 100ml. (ie. 100ul to 100ml and 200ul to 200ml bottles).
NB	1M MgSO ₄ at 1ul/1ml in the M9 buffer gives a final concentration of 1mM

3

The autoclave is SYCHEM VS Series. The media cycle is 15 minutes at 121 degrees centigrade. It is then cooled down in the machine for 3 hours.

3h 15m



- 4 After autoclaving, add 100 μ l 1M MgSO_4 per 100 ml. (ie. 100 μ l to 100 ml and 200 μ l to 200 ml bottles).

10m

NB: 1M MgSO_4 at 1 μ l/1ml in the M9 buffer gives a final concentration of 1 mM