Upload image

2019

Working

Preparing 10 L of M9 buffer for nematode culture

Gurdon Institute media kitchen[1]

[1]Cancer Research UK / Wellcome Gurdon Institute

dx.doi.org/10.17504/protocols.io.3kngkve

Cristian Riccio

ABSTRACT

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

MATERIALS

NAME	CATALOG #	VENDOR
double distilled water (ddH2O)		
Di-Sodium Hydrogen Orthophosphate Dihydrate Certified AR for Analysis Fisher Chemical	S/4450/53	
Sodium chloride meets analytical specification of Ph.Eur Fisher Chemical	S/3160/65	Fisher Scientific
Magnesium Sulfate Heptahydrate Certified AR for Analysis Fisher Chemical	M/1050/53	Fisher Scientific
Potassium Dihydrogen Orthophosphate Certified AR for Analysis Fisher Chemical	P/4800/53	Fisher Scientific
SYCHEM autoclave	View	Syschem

SAFETY WARNINGS

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

20m

1

Ingredients	Quantity	
Na2HPO4		60g
KH2PO4		30g
NaCl		50g
Double distilled H2O	up to 10L	
Add 1M MgSO4 after autoclaving @ 100ul/100ml		

M9_buffer_10L.xls

15m

2

1	Dissolve ingredients in 8L double distilled H2O
2	Adjust volume to10L using double distilled H2O
3	Measure or use pump to dispense aliquots accurately.
4	Label, date and autoclave.

5	After autoclaving, add 100ul 1M MgSO4 per 100ml. (ie. 100ul to 100ml and 200ul to 200ml bottles).
NB	1M MgSO4 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 µl 1M MgSO4 per 100 ml. (ie. 100 µl to 100 ml and 200 µl to 200 ml bottles). 10m

NB: 1M MgSO4 at 1 µl/1ml in the M9 buffer gives a final concentration of 1 mM