Media Recipes

Matthew Sullivan

Abstract

Citation: Matthew Sullivan Media Recipes. **protocols.io**
dx.doi.org/10.17504/protocols.io.deq3dv
Published: 21 Jan 2016

Guidelines

SN Media Recipe

1. Pour a 75% Seawater mixture (ie. for **1L**, add **750ml White Point Seawater** and **250ml MQ Water**) into a clean, capped bottle. Autoclave using the p18 liquid cyle (NOTE: This should take about 2 hours).

2. Let your 75% Seawater cool to room temperature. (NOTE: To speed up the process, put your 75% Seawater in the cold room).

3. Add nutrients to your cooled 75% Seawater to create the final **SN Media** as detailed below:

Amount 75% Seawater	**1000mL (1L)**	**500 mL**	**250 mL**	**100 mL**
EDTA (1g/L)	5.75mL	2.875mL	1.45mL	0.75mL
K_2HPO_4 (6.1g/L)	2.6mL	1.3mL	0.65mL	0.325mL
$NaNO_3$ (30%)	2.6mL	1.3mL	0.65mL	0.325mL
Na_2CO_3 (4g/L)	2.6mL	1.3mL	0.65mL	0.325mL
CTMM (Trace Metal)	1mL	0.5mL	0.25mL	0.125mL
Vitamin B12 (10mg/mL)	0.1mL (100µL)	0.05mL (50µL)	0.025mL (25µL)	0.0125mL (12.5µL)

4. Store the **SN Media** outside of direct UV light (eg. sunlight).

SN Media + NH_4 Recipe

1. Pour a **75% Seawater** mixture (ie. for **1L**, add **750ml White Point Seawater** and **250ml MQ Water**) into a clean, capped bottle. Autoclave using the p18 liquid cyle (NOTE: This should take about 2 hours).

2. Let your 75% Seawater cool to room temperature. (NOTE: To speed up the process, put your 75% Seawater in the cold room).

3. Add **nutrients** to your cooled 75% Seawater to create the final **SN Media + NH_4** as detailed

below:

Amount 75% Seawater	1000mL (1L)	500 mL	250 mL	100 mL
EDTA (1g/L)	5.75mL	2.875mL	1.45mL	0.75mL
K_2HPO_4 (6.1g/L)	2.6mL	1.3mL	0.65mL	0.325mL
$NaNO_3$ (30%)	2.6mL	1.3mL	0.65mL	0.325mL
Na_2CO_3 (4g/L)	2.6mL	1.3mL	0.65mL	0.325mL
CTMM (Trace Metal)	1mL	0.5mL	0.25mL	0.125mL
Vitamin B12 (10mg/mL)	0.1mL (100µL)	0.05mL (50µL)	0.025mL (25µL)	0.0125mL (12.5µL)
1.6M NH_4Cl	2.6mL	1.3mL	0.65mL	0.325mL

4. Store the **SN Media + NH_4** outside of direct UV light (eg. sunlight).

50% AMP / 50% SN Media Recipe

1. Make **1L Turk's Island Salt Mix** in a clean, capped bottle as per below:

Chemical	g/1000mL (1L)	g/2000ml (2L)
NaCl	28.11	56.22
$MgSO_4$-$7H_2O$	6.9	13.8
$MgCl_2$-$6H_2O$	5.49	10.98
$CaCl_2$-$2H_2O$	1.47	2.94
KCl	0.67	1.34
MQ Water	q. to 1000mL	q. to 2000mL

2. Autoclave Turk's Island Salt Mix using the p18 liquid cycle (NOTE: This should take about 2 hours.) Let your Turk's Island Salt Mix cool to room temperature. (NOTE: To speed up the process, put your Turk's Island Salt Mix in the cold room).

3. Add the **nutrients, buffers, and trace metals** to your cooled Turk's Island Salt Mix to create your **100% AMP1 Media.** (NOTE: $NaHCO_3$ needs to be made fresh each time)

Reagent	g/1000mL (1L)	g/2000mL (2L)
	Nutrients	
0.1M NaH_2PO_4-H_2O	500µL	1000µl (1mL)
0.8M $(NH_4)_2SO_4$	500µl	1000µl (1mL)
	Buffers	
0.6M $NaHCO_3$ (Needs to be made fresh)	10 mL	20 mL
1M HEPES	1 ml	2 ml
	Trace Metals	

Stock Trace Metal Mix (NOT the same CTMM used with SN Media and PRO99 Media)	10µl	20µl

4. Mix your 100% AMP1 media with SN Media in a 1:1 ratio (ie. for 1L, mix **500mL 100% AMP1** with **500mL SN Media**) in a clean, autoclaved bottle. (Optional: Autoclave on p13 liquid cycle to ensure sterility of media). This is your **50% AMP / 50% SN Media.**

5. Store your 50% AMP / 50% SN Media outside of direct UV light (eg. sunlight).

Pro99 Media Recipe

1. Pour a **100% SIO, Bigelow or Sargasso Seawater** into a clean, capped bottle. Autoclave using the p18 liquid cycle (NOTE: This should take about 2 hours.)

2. Let your 100% Seawater cool to room temperature. (NOTE: To speed up the process, put your 75% Seawater in the cold room).

3. Add nutrients to your cooled 100% Seawater to create the final **Pro99** as detailed below:

Amout 100% Seawater	**1000mL (1L)**	**500mL**	**250mL**	**100mL**
1.6M NH_4Cl	500µl	250µl	125µl	50µl
0.1M Na_2HPO_4	500µl	250µl	125µl	50µl
CTMM (Trace Metal)	100µl	50µl	25µl	10µl

4. Store the **Pro99 Media** outside of direct UV light (eg. sunlight).

Top Agar and Bottom Agar Recipe

Add the following amounts of **low melting point agarose (LMP Agarose)** to your pre-made media (previously made using the aforementioned directions) and autoclave on a p13 liquid cycle or microwave until dissolved.

Media Volume	**1000mL (1L)**	**500mL**
Top Agar	5.5g LMP Agarose	2.75g LMP Agarose
Bottom Agar	5.0g LMP Agarose	2.50g LMP Agarose

Protocol