Jun 15, 2021

Ney's Spring Media Preparation

Annette Rowe[1], Leah Trutschel[1]

[1]University of Cincinnati

dx.doi.org/10.17504/protocols.io.bqjgmujw

Rowe Lab

Leah Trutschel

ABSTRACT

This is for protocol for making Ney's Spring high pH media and plates. These consist of a basal minimal media with differing electron donors and acceptors depending on what organism is targeted.

This includes instructions for:
sulfur/acetate plates & media
Methanol plates & media
Sulfate/acetate plates & media

DOI

dx.doi.org/10.17504/protocols.io.bqjgmujw

PROTOCOL CITATION

Annette Rowe, Leah Trutschel 2021. Ney's Spring Media Preparation . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bqjgmujw

CREATED

Dec 09, 2020

LAST MODIFIED

Jun 15, 2021

PROTOCOL INTEGER ID

45384

SAFETY WARNINGS

These are high pH medias and gloves should be worn during their preparation and disposal. Addition of sulfide and polysufide should be performed in a fume hood.

Ney's Spring Basal Salts

1 These are the basal salts used in all medias. If making liquid media, add salts to 700mL milli Q water. If making plates, then start with 400 mL Milli Q (additional components will then be added to bring up to 1L later)

A	B	C	D
Component	MW (g/mol)	Amount added (g)	Concentration (mM)
NaCl	58.44	11.7	200
Na2SO4	142.04	0.284	2
NH4Cl	53.49	0.535	10
MgCl2 * 6H2O	203.3	0.102	0.5

Components added from Stock

2 These are all prepared as stock solutions first, then are added to the media after the basal salts. This should bring the liquid media up to ~1L, and media to be used for plates to ~700 mL. After adding these, check the pH. Media should be between pH 10-11. Aim for closer to 10.5 for plates, while liquid media can be closer to 11.

For plates, prepare a second solution of 300 mL milli Q water with 15g **NOBLE** agar. Regular agar burns incredibly easy at high pH and won't be sufficient for this media. Add a stir bar and autoclave separately from the salt solution

A	B	C	D	E	F	G
Component	MW (g/mol)	Amount of Stock prepared	Amount added to stock (g)	Concentration (mM)	amount added to media	Final conc.
K2HPO4	174.18	10X (500mL)	0.871	10	100 mL	1 mM
Na2CO3	105.99	10X (500 mL)	53	1000	100 mL	100 mM
CAPS (N-cyclohexyl-3-aminopropanesulfonic acid)	221.32	10X (500 mL)	11	100	100 mL	10 mM
CH3COONa (sodium acetate)	136.08	100X (200 mL)	5.44	200	10 mL	2 mM
NaOH	39.99	100X (200 mL)	4	500	10 mL	5 mM

***Please check for each electron donor/acceptor combo if it it is added before or after autoclaving as they all differ**

3 **Additions after autoclaving:**

Once media has cooled you may also add vitamins and minerals (1mL of each from a 10,000X conc. Recipes for these can be found here:).

Note for plates:
*It is very important to let the media cool almost entirely before any more additions! Plates especially will burn and turn black almost immediately if your media is still hot when you combine the 700mL salt solution and 300 mL agar solution. Wait until just warm and then very slowly pour the basal salt solution into the agar solution, stirring the entire time.

Note for anaerobic media:
*While liquid media is still hot and fresh out of the autoclave, immediately begin purging with N_2 gas. Purge until cool, then you may add vitamins, minerals, and other components.

Electron donor & Acceptor variants

4 ***Check first to see if whether these need to be added before or after autoclaving!!

Methylotroph/Methanogen enrichment

Citation: Annette Rowe, Leah Trutschel (06/15/2021). Ney's Spring Media Preparation . https://dx.doi.org/10.17504/protocols.io.bqjgmujw

A	B	C	D	E	F
Component	MW (g/mol)	Stock	Amount added for stock (g)	Concentration	Amount added to media
MeOH	32.04	Pure MeOH	-	2.5 mM	2.5 mL
Na2S * 9H2O (sodium Sulfide)	240.18	100 mM	2.4	1 mM	10 mL

*Add methanol and sulfide after autoclaving at the same time as vitamins and minerals
** Sodium sulfide is optional but can be added to anaerobic media to help with oxygen scavenging.
*** MeOH plates will be clear, while media will be mostly clear but may form a dark green/turquoise precipitate

Sulfate Reducers Enrichment

A	B	C	D
Component	MW (g/mol)	Amount added	Concentration (mM)
NaSO4 (Sodium sulfate)	142.04	1.42 g	10
CH3COONa (sodium acetate)	136.08	0.82 g	10

*These components can be added at the same time as components in the basal salts for a final total of 12 mM of $NaSO_4$ and CH_3COONa
**After autoclaving, 1mM Na_2S*9H_2O may also be added to anaerobic media to help with oxygen scavenging.
*** Sulfate plates will be clear, while media will be mostly clear but may form a dark green/turquoise precipitate

Sulfur reducers/oxidizers Enrichment

A	B	C	D
Component	MW (g/mol)	Amount added	Concentration (mM)
Polysulfide	-	8.8 mL	20mM
CH3COONa (sodium acetate)	136.08	0.41g	5mM

*Acetate can be added at the same time as components in the basal salts for a final total of 7 mM.
**Polysulfide prepared according to Moser & Nealson 1996, "Growth of the Facultative Anaerobe *Shewanella putrefaciens* by Elemental Sulfur Reduction"
***Add polysulfide once media is almost entirely cool using a syringe. Will immediately turn a very dark olive "bottle glass" green and have a very strong sulfur smell. As the polysulfide oxidizes (I recommend placing them in a fume hood for at least 24 hours) the plates will turn a translucent pale blue rather than the typical solid white of elemental sulfur plates.

Citation: Annette Rowe, Leah Trutschel (06/15/2021). Ney's Spring Media Preparation . https://dx.doi.org/10.17504/protocols.io.bqjgmujw