bjmhkk36

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TAP media preparation V.(bjmhkk36)

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This protocol describes the preparation of TAP media. Usually used for growing algae cells, as Chlamydomonas reinhardtii. The protocol is derived from the protocol descrived at https://www.chlamycollection.org/methods/media-recipes/tap-and-tris-minimal/.

[Gorman, D.S., and R.P. Levine (1965) Proc. Natl. Acad. Sci. USA 54, 1665-1669]

EXTERNAL LINK

https://www.chlamycollection.org/methods/media-recipes/hutners-trace-elements/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Molino JVD, Carvalho JCMd, Mayfield SP (2018) Comparison of secretory signal peptides for heterologous protein expression in microalgae: Expanding the secretion portfolio for Chlamydomonas reinhardtii. PLoS ONE 13(2): e0192433. doi: 10.1371/journal.pone.0192433

PROTOCOL CITATION

Joao Vitor Molino 2020. TAP media preparation. **protocols.io** https://protocols.io/view/tap-media-preparation-bjmhkk36

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Molino JVD, Carvalho JCMd, Mayfield SP (2018) Comparison of secretory signal peptides for heterologous protein expression in microalgae: Expanding the secretion portfolio for Chlamydomonas reinhardtii. PLoS ONE 13(2): e0192433. doi: 10.1371/journal.pone.0192433

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KEYWORDS

Microalgae, Recombinant, electroporation, plasmid, Media, Algae, Chlamydomonas reinhardtii

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40329

GUIDELINES

All steps described in this protocol are intended to be conducted in a research laboratory.

SAFETY WARNINGS

Use EPIs at all times.
Concentrated acetic acid solution used during preparation.

DISCLAIMER:

BEFORE STARTING

- Prepare stock solutions
- Separate flasks to distribute the prepared media for autoclavation
- Separate magnetic stirrer for mixing
- Large enough becker for media preparation
- Pippetes/volume measuring apparatus for components

Components mixing

1

1. Add approximatelly [M]**90 % volume** of ddH_20 to a large enough vessel.
2. Add a magnetic bar and start and keep mixing with a magnetic stirrer during the preparation.
3. Place pH probe electrode in the solution for pH monitoring

For **1 L final media volume**

1. Add **10 mL 2M Tris base (e.g. Trizma)**
2. Add **10 mL Solution A**
3. Add **1 mL Phosphate solution**
4. Add **1 mL Hutner`s trace solution**
5. Add **1 mL Glacial acetic acid** , then add drops until pH**7** is reached
6. Stop mixing, and add dd H_20 until **1 L final volume** is reached
7. Start mixing again until complete mixing is achieved. (5 minutes should suffice).

Componets concentration and informations.

Stock solution	Component	Amount for Stock (g) *Check final volume for each in column A	Molecular weight (g/mol)	Final media concentration (mM)
Solution A (1000ml)	NH4Cl	40	53.49	7.48
	MgSO4 . 7H20	10	246.47	0.406
	CaCl2 . 2H2O	5	147.01	0.34
Phosphate solution	K2HPO4	27	174.2	0.620
(250mL)	KH2PO4	14	136.086	0.41
Tris Solution (1000mL)	Tris	242.28	121.14	20
Acetic acid	Acetic Acid Glacial		60.05	~17.5

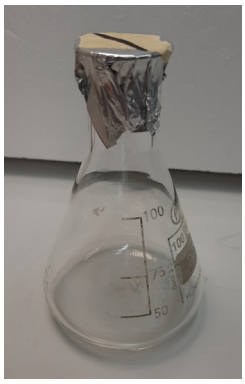
Hutner`s trace composition and protocol for preparation can be found here.

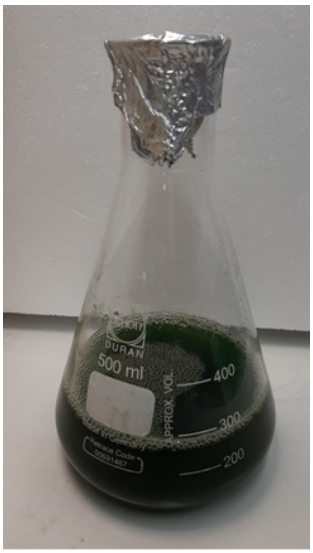
Liquid transfer

2 1. Transfer the newlly prepared media to flasks for autoclavation

Typically:

- 1L flasks with blue caps
- Erlenmeyers with a max volume capacity of 100 mL are filled with 50 mL, capped with alluminium foil (2 layers)
- Erlenmeyers with a max volume capacity of 500 mL are filled with 250 mL, capped with alluminium foil (2 layers)





Autoclavation

3 1. Place flasks inside the autoclave (For flasks with lid, make sure it is loosen enough to allow vapor passage)
2. Set autoclavation to **121 °C** for at least **00:15:00** , 15 psi.
3. After autoclavation, wait media to cool down and it is ready for use.