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TB Media Preparation

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We use this protocol and it's working

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Disclaimer

No conflict of interest.

Abstract

Terrific Broth (TB) as described by Tartoff, K.D. and Hobbs, C.A. (1987) is a nutritionally rich medium, with higher concentrations of peptone, yeast extract, and glycerol as a carbon source (**Thermo Scientific | Terrific Broth**). It supports high cell titers (**Wood et al. 2017**), and subsequent yields for plasmid DNA (**Wood et al. 2017**). While it has been referenced often we could not find the original Tartoff, K.D. and Hobbs, C.A. (1987) publication. What we present here has been passed down by oral tradition. We had sadly no access to the original publication.
Here we describe the steps we take to prepare the two solutions, denoted here as solution 1 and 2. Each is prepared and autoclaved separately before being combined in a 1:10 ratio shortly before inoculation with bacteria and antibiotics. The first solution contains all nutritional components, while the second has buffer characteristics, probably allowing a constant pH during the growth of the bacteria.

Image Attribution

Exotoxins Lab (TM)

Guidelines

- As good microbiological practice, all containers used for components should be labeled with the name of the solution, the date of creation / aliquote, and initials of the person who did it, in order to ask questions if something is needed.
- If you have doubts about how to perform a step in the protocol, consult your supervisor or someone who has prepared the solution before.
- For any questions, feel free to email us (exotoxinslab_(at)_gmail.com). We are more than happy to help you.
- Some of the protocols will be posted in our YouTube channel (**www.youtube.com/@exotoxinslab**).

Materials

Materials

- Peptone / Tryptone from Casein **Carl Roth Catalog #**8952
- Glycerol **Carl Roth Catalog #**3783
- Distilled Water (dH_2O)
- Yeast Extract **Carl Roth Catalog #**2363.1

Equipment

Laboratory bottles borosilicate glass	NAME
Glassware	TYPE
Simax	BRAND
FL15	SKU
https://www.laborversand.de/product_info.php?info=p477_Laboratory-bottles-SIMAX-sup-----sup-.html&XTCsid=nrv1449jacsrd2h98p92utcr93	LINK
Laboratory bottles made of clear borosilicate glass 3.3 With white imprinted graduation, completely autoclavable With attached blue screw cap made of PP and blue pouring ring made of PP, in acc. with ISO 4796-1 Height is with attached screw cap!	SPECIFICATIONS

Solution 1:

	A	B	C	D
	dH2O	996 mL	498 mL	249 mL
	Glycerol	4 mL	2 mL	1 mL
	Tryptone	12 g	6 g	3 g
	Yeast extract	24 g	12 g	6 g
		1 L	500 mL	250 ML

Materials solution 1 for 1 L, 0.5 L and 0.2

Solution 2:

	A	B	C
		100 mL	final c (10x)
	Kaliumdihydrogenphosphate (KH2PO4)	2.32 g	0.17 M
	Dikaliumhydrogenphosphate (K2HPO4)	16.4 g	0.72 M
	dH2O	100 mL	

Materials solution 2 (10x) for 100 mL

Protocol materials

- Distilled Water
- Glycerol **Carl Roth Catalog #**3783
- Peptone / Tryptone from Casein **Carl Roth Catalog #**8952
- Yeast Extract **Carl Roth Catalog #**2363.1

Safety warnings

The process of autoclaving requires the use of high temperatures and specialized equipment. Make sure you have been instructed in the use of the autoclave.
When handling recently autoclaved liquids, their temperatures can be above 60°C, and can cause severe burns. Follow the safety protocols of your institution and use always personal protective equipment.

Before start

- Be aware of your lab's safety protocols and check the attached Warnings (Guidelines and Warnings) to see what steps are necessary to protect yourself, such as protocol-appropriate personal protective gear like lab coat, gloves, and glasses.
- Make sure to read the whole protocol before starting and have all the materials at hand.
- Before using any machine, make sure you have been properly instructed in its use, and read the Standard Operating Procedures (SOP) with the safety information provided by your institution or supervisor.

Preparation solution 1

1 For 1 L of solution pour about 500 mL of Distilled Water into a measuring cylinder

2 Add 12 g of Peptone / Tryptone from Casein **Carl Roth Catalog #**8952

3 Add 24 g of Yeast Extract **Carl Roth Catalog #**2363.1

4 Add 4 mL of Glycerol **Carl Roth Catalog #**3783

5 Add a magnetic rod to the cylinder and mix on a magnetic stirrer, until fully dissolved

6 Pour an additional 500 mL Distilled Water for a final volume of 1 L

7 Evenly distribute 500 ml of the solution into 2× 1 L bottles / flasks (see Materials for details). To avoid the explosion of the bottles during autoclaving, leave the lid loose and secure it with autoclave tape.

8 Autoclave at 121 °C at (psi) for 15 - 20 min. Make sure you know how to use the autoclave before you use it.

Preparation of solution 2

9 For 100 mL of solution pour about 50 mL of Distilled Water into a measuring cylinder

10 Add 2.32 g KH_2PO_4

11 Add 16.4 g K_2HPO_4

12 Add a magnetic rod/fish to the cylinder, and mix on a magnetic stirrer, until fully dissolved

13 Pour the solution into a 200 ml bottle. To avoid the explosion of the bottles during autoclaving, leave the lid loose and secured with autoclave tape.

14 Autoclave at 121 °C at (psi) for 15 - 20 min. Make sure you know how to use the autoclave before you use it. Do not autoclave together with solution 1. The difference in amounts (200 ml vs 500 ml) will cause a overheating in solution 2, if the temperature sensor is evaluating the 500 ml volume temperature.

Combining the solutions

15 After the solutions are autoclaved, **close the lids tight**, and store them at room temperature. Just before use, add solution 2 to solution 1 for a final concentration of [M] 10 % (v/v) of solution 2 .

Protocol references

Tartoff KD, Hobbs CA. Improved media for growing plasmid and cosmid clones. Bethesda Res Lab Focus. 1987;9:12.

Wood WN, Smith KD, Ream JA, Lewis LK. Enhancing yields of low and single copy number plasmid DNAs from Escherichia coli cells. J Microbiol Methods. 2017 Feb;133:46-51. doi: 10.1016/j.mimet.2016.12.016. Epub 2016 Dec 23. PMID: 28024984; PMCID: PMC5286560.

Terrific Broth | Thermo Scientific

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