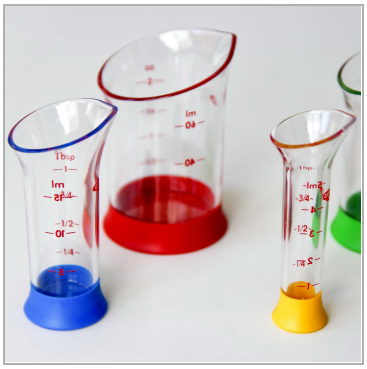

VERSION 3

FEB 09, 2023

OPEN ACCESS

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We use this protocol and it's working

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Terrific broth (TB) medium V.3

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ABSTRACT

IBI's Terrific Broth is used with Glycerol in cultivating recombinant strains of *E. coli*. Terrific broth is a highly enriched medium for improving yield in plasmid bearing *E. coli*. Recombinant strains have an extended growth phase in the medium. The addition of tryptone and yeast extract in the medium will allow higher plasmid yield per volume. Glycerol is used as a carbohydrate source in this formulation. Unlike glucose, glycerol is not fermented to acetic acid.

GUIDELINES

Follow step by step, unless stated otherwise. Equipment needed should be standard to a microbiology lab.

MATERIALS

Analytical scale, autoclave, bottle(s), weight vessel, LAF bench

SAFETY WARNINGS

You can mix Dextrose from the beginning with the other compounds, and autoclave together. While this is more time efficient and easier, it is important to take into account the possibility of toxic byproducts produced by the Millard reaction when autoclaving, producing Acrylamide, a probable human carcinogen (IARC Group 2A). Furthermore, when removing autoclaved components, be sure to take care as these can be very hot. If using antibiotics, use sufficient PPE to protect yourself, as some can be toxic to humans.

BEFORE START INSTRUCTIONS

Prepare glassware by cleaning it, and ensure that scale is sufficiently calibrated

Terrific broth base solution

20m

1 Fill the bottle with 200 mL distilled water

> **Note**
>
> Consider making a larger batch of 5 bottles of base solution at once and use all Potassium phosphate solution and carbon solution at once to get 2.5 L of medium

2 Measure 11.8 g Yeast extract and 5.9 g Tryptone. Add 7.5 g for making agar plates

Materials:

- Yeast Extract **Sigma-aldrich Catalog #Y0875**
- Tryptone **Millipore Catalog #T9410**
- Agar **Sigma-aldrich Catalog #A1296**

3 Add powdered solids into bottle, and use a magnetic mixer with a stir bar to mix for 00:05:00 5m

4 Add 100 mL Potassium phosphate buffer (1 M, 6.2 pH)

> **Protocol**
>
> 
>
> NAME
>
> **Potassium phosphate buffer (1.0 M, pH 6.2)**
>
> CREATED BY
>
> **Andreas Sagen**
>
> PREVIEW

5 Adjust pH while mixing to pH 7.2 using concentrated sodium hydroxide

6 Add distilled water to a total of 400 mL

7 Autoclave liquid at 121 °C for 00:15:00 15m

Terrific broth carbon solution

8 Fill the bottle with 300 mL double-distilled water

9 Add 20 mL Glycerol and mix solution well

Materials:
Glycerol **MP Biomedicals Catalog #194680**

10 Adjust pH while mixing to pH 7.2 using concentrated sodium hydroxide

11 Add distilled water to a total of 500 mL

12 Autoclave liquid at 121 °C for 00:15:00 Autoclave liquid at 121 °C for 00:15:00 15m

500 mL Terrific broth

13 Add 100 mL carbon solution to base solution

14 Aliquot medium in 100 mL units, and store refrigerated (4 °C)

Note

If there is no space to store refrigerated, keep sterile and store at room temperature