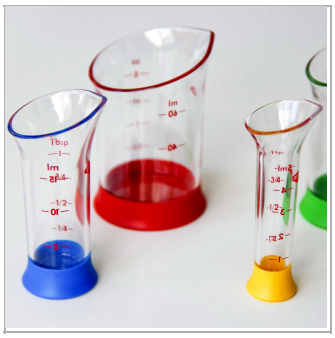

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Terrific broth (TB) medium

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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

500 mL Terrific broth

1 Create a base solution consistent of Tryptone, Yeast extract, Potassium phosphate monobasic and Potassium phosphate dibasic in a 500 mL Reagent Bottle

1.1 Fill the bottle with 300 mL double-distilled water 1m

1.2 Measure 11800 mg Yeast extract, 5900 mg Tryptone, 1100 mg Potassium phosphate monobasic and 4700 mg Potassium phosphate dibasic 5m

Powdered compounds:
Yeast Extract **Sigma-aldrich Catalog #Y0875**
Tryptone **Millipore Catalog #T9410**
Potassium phosphate monobasic **Sigma-aldrich Catalog #P5379**
Potassium phosphate dibasic **Sigma-aldrich Catalog #P8281**

1.3 Add powdered solids into bottle, and use a magnetic mixer with a stir bar to mix for 00:05:00 10m

1.4 Adjust pH while mixing to pH 7.2 using concentrated sodium hydroxide 2m

1.5 Autoclave liquid at 121 °C for 00:15:00 30m

2 Create a carbon solution consistent of glycerol in a 100 mL Reagent Bottle

2.1 Fill the bottle with 60 mL double-distilled water 1m

2.2 Add 4 mL Glycerol and mix solution well 1m

Liquid compounds:
Glycerol **Sigma-aldrich Catalog #G7757**

2.3 Adjust pH while mixing to pH 7.2 using concentrated sodium hydroxide 2m

2.4 Add double-distilled water to a total of 100 mL 1m

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

3 After having prepared a base solution and carbon solution, combine the sterile solutions. All steps from this point take place in a LAF bench, to prevent contamination

> **Note**
>
> Remember allow flow to stabilize for minimum 15 minutes before use and clean LAF bench before use to prevent contamination of medium stock

3.1 Add 100 mL carbon solution to base solution, and mix well 1m

3.2 Add sterile water to a total of 500 mL 1m

> **Note**
>
> Cool to 50°C and supplement with antibiotics as appropriate