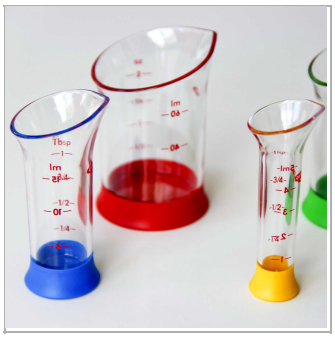

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We use this protocol and it's working

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Lysogeny Broth (LB) medium V.2

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ABSTRACT

Lysogeny broth (LB) is a nutritionally rich medium which is primarily used for the growth of bacteria[1]. The initialism is also commonly, albeit incorrectly, taken to mean **Luria broth**, **Lennox broth**, or **Luria-Bertani medium**. According to its creator Giuseppe Bertani, the abbreviation **LB** was actually intended to stand for **lysogeny broth**. The formula of the **LB medium** was published in 1951 in the first paper of Bertani on lysogeny[2]. There are several common formulations of **LB**. Although they are different, they generally share a somewhat similar composition of ingredients used to promote growth, including the following: Peptides and casein peptones, Vitamins (including B vitamins), Trace elements (e.g. nitrogen, sulfur, magnesium) and Minerals.
Sodium ions for transport and osmotic balance are provided by sodium chloride (NaCl). Tryptone is used to provide *essential amino acids* such as peptides and peptones to the growing bacteria, while the yeast extract is used to provide a plethora of organic compounds helpful for bacterial growth. These compounds include vitamins and certain trace elements.

GUIDELINES

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

MATERIALS

Analytical scale, autoclave, bottle, weight vessel, LAF bench

SAFETY WARNINGS

When removing autoclaved components, be sure to take care as this 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

1 All compounds are measured using a high precision analytical scale from powdered compounds. Each compound is measured to within 1% of the target weight. All compounds are mixed in a Duran bottle

500 mL LB-Lennox (broth) medium

1.1 Fill the bottle with 400 mL double-distilled water

1.2 Measure 5000 mg Tryptone, 2500 mg Yeast extract and 2500 mg Sodium chloride

Powdered compounds:
Tryptone **Millipore Catalog #T9410**
Yeast Extract **Sigma-aldrich Catalog #Y0875**
Sodium chloride **Sigma-aldrich Catalog #S9625**

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

1.4 Adjust pH while mixing to pH 6.7 using concentrated sodium hydroxide

1.5 Add distilled water to a total of 500 mL

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

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

2 All compounds are measured using a high precision analytical scale from powdered compounds. Each compound is measured to within 1% of the target weight. All compounds are mixed in a Duran bottle

500 mL LB-Lennox (agar) medium

2.1 Fill the bottle with 400 mL double-distilled water

2.2 Measure 5000 mg Tryptone, 2500 mg Yeast extract, 2500 mg Sodium chloride and 7500 mg agar

Powdered compounds:
Tryptone **Millipore Catalog #T9410**
Yeast Extract **Sigma-aldrich Catalog #Y0875**
Sodium chloride **Sigma-aldrich Catalog #S9625**
Agar **Sigma-aldrich Catalog #A1296**

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

2.4 Adjust pH while mixing to pH 6.7 using concentrated sodium hydroxide

2.5 Add distilled water to a total of 500 mL

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

Note

Cool to 50°C and supplement with antibiotics as appropriate

Agar can be stored, then reheated to 50°C to be poured