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Lysogeny Broth (LB) medium V.1

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I use this protocol and it's working

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Abstract

Lysogeny broth (LB) is a nutritionally rich medium which is primarily used for the growth of bacteria[1]. LB broth is commonly used when cultivating Escherichia coli. There exist different formulations of LB and lead to the development of derivations for specialized use.

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

Protocol materials

- Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9625
- Tryptone **Merck Millipore (EMD Millipore) Catalog #**T9410
- Yeast Extract **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Y0875
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- Yeast Extract **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Y0875
- Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9625
- Agar **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A1296

Troubleshooting

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

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

500 mL LB-Lennox (broth) medium

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

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 **Merck Millipore (EMD Millipore) Catalog #**T9410
Yeast Extract **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Y0875
Sodium chloride **Merck MilliporeSigma (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

500 mL LB-Lennox (agar) medium 20m

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

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 **Merck Millipore (EMD Millipore) Catalog #**T9410
Yeast Extract **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Y0875
Sodium chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9625
Agar **Merck MilliporeSigma (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