

LB Medium with Kanamycin Preparation Protocol

Formulation:

- Tryptone: 10 g/L
- Yeast Extract: 5 g/L
- Sodium Chloride (NaCl): 10 g/L
- Distilled Water: (900mL)
- Kanamycin (stock solution, typically 50 mg/mL)

Procedure:

1. **Weighing the Components:**
 - Weigh 10 grams of Tryptone.
 - Weigh 5 grams of Yeast Extract.
 - Weigh 10 grams of Sodium Chloride (NaCl).
2. **Dissolving the Components:**
 - Add the Tryptone, Yeast Extract, and Sodium Chloride to a beaker or flask.
 - Add approximately 900 mL of distilled water to the beaker.
 - Stir the solution until all components are completely dissolved.
3. **Adjusting the Volume:**
 - After the components are dissolved, adjust the final volume of the solution to 1 liter using distilled water.
4. **Sterilization:**
 - Transfer the prepared LB medium to suitable containers (e.g., bottles or flasks).
 - Autoclave the medium at 121°C for 15-20 minutes to sterilize.
5. **Cooling:**
 - Allow the sterilized LB medium to cool to about 50-55°C (not fully cooled to room temperature but cool enough not to degrade the antibiotic).
6. **Adding Kanamycin:**
 - Once the medium has cooled, add kanamycin to a final concentration of 50 µg/mL.
 - For a 1 liter solution, add 1 mL of kanamycin stock solution (50 mg/mL).
 - Mix thoroughly to ensure even distribution of the antibiotic.
7. **Storage:**
 - The LB medium with kanamycin can be stored at room temperature or in a refrigerator until needed. If making LB agar plates, pour the medium into petri dishes immediately after adding kanamycin.

Optional:

- If solid LB agar plates are required, add 15 g/L of agar before autoclaving. After autoclaving, cool to about 50-55°C before adding kanamycin and pouring into plates.

This protocol yields 1 liter of LB medium with kanamycin. Adjust the quantities proportionally for different volumes.

UMass Used: FisherSci LB broth (BP1426-2)

Link:

<https://www.fishersci.com/us/en/catalog/search/products?keyword=LB+broth+%28BP1426-2%29>

I am using materials from Amazon!