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# Selective King's B Medium for Gram Negative Bacteria

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**Protocol status:** Working
**We use this protocol and it's working**

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## Disclaimer

This protocol has been tested in a standard microbiology laboratory setting. Users must ensure that all safety precautionsare followed, including the use of sterile techniques, proper antibiotic handling, and appropriate biosafety measures. Cycloheximide is toxic and must be handled with care. Ensure compliance with institutional biosafety regulationswhen culturing bacteria, especially if working with pathogenic or genetically modified strains. The authors are not responsible for any misuse, contamination, or safety violations resulting from the improper execution of this protocol.

## Abstract

King's B (KB) medium is a nutrient-rich selective medium primarily used for the isolation and growth of fluorescent *Pseudomonas* species, particularly Gram-negative bacterium. This protocol describes the preparation of KB medium, including the addition of antibiotics to suppress unwanted microbial growth. Cycloheximide (100 mg/mL) is incorporated to inhibit fungal contamination, while cephalexin (10 mg/mL) is used to retard the growth of Gram-positive bacteria, ensuring selective enrichment of Gram-negative bacteria such as *Pseudomonas*. The procedure involves media preparation, sterilization, antibiotic addition, and plate pouring under sterile conditions. The expected outcome is a selective growth medium that promotes the recovery and study of *Pseudomonas*species, particularly those producing fluorescent pigments, which can be visually detected under UV light.

## Guidelines

- Ensure all glassware and equipment are sterile before use.
- Use a weigh boat to accurately measure dry ingredients.
- Do not add antibiotics before autoclaving to prevent degradation.
- When adding antibiotics, work in a biosafety cabinet to maintain sterility.
- After pouring agar plates, let them solidify at room temperature before sealing and storing.
- Store prepared plates at 4°C in sealed bags to prevent moisture loss.

## Materials

### Glassware & Equipment

- 1L Autoclavable Bottle – For preparing the base medium
- 250 mL Autoclavable Bottle – For boric acid solution
- Magnetic Stir Bar & Stir Plate – For thorough mixing
- 15 mL sterile Falcon Tubes® and 1L Beaker – For preparing antibiotics
- Autoclave – For sterilization
- Pipettes & Tips (Filter Sterile) – For accurate liquid handling
- Petri Dishes – For plating solid medium
- Biosafety Cabinet or Sterile Hood – For antibiotic addition and plate pouring

### Chemicals & Reagents

- Proteose Peptone – 15 g
- Potassium Phosphate Dibasic ($K_2HPO_4$) – 1.5 g
- Magnesium Sulfate ($MgSO_4$) – 0.74 g
- 100% Glycerol – 10 mL
- Agar (for solid medium only) – 15 g
- Boric Acid – 1.5 g
- Cycloheximide – 200 mg (antifungal agent)
- Cephalexin – 80 mg (antibiotic)
- Deionized Water (DI) – To prepare media

## Safety warnings

- Cycloheximide is toxic—avoid inhalation, ingestion, and skin contact. Dispose of properly.
- Cephalexin may cause allergic reactions—handle with care, especially if sensitive to β-lactam antibiotics.
- Autoclaved media is extremely hot—handle with heat-resistant gloves to prevent burns.
- All bacterial cultures must be handled under appropriate biosafety levels (BSL-1 or BSL-2, depending on the organism used).
- Dispose of used media and cultures by autoclaving before discarding to prevent contamination.

## Ethics statement

This protocol involves standard microbiological techniques and does not require ethical approval.

## Before start

- Ensure the autoclave is functioning properly and set at 121°C, 15 psi for 15 minutes.
- Verify that all reagents and chemicals are fresh and within expiration dates.
- Work in a clean, sterile environment to avoid contamination.
- Label all bottles and plates properly with medium type, preparation date, and initials.
- When handling cycloheximide, wear gloves and a lab coat to avoid exposure.

## Procedures

1 **Prepare the Base Medium**

In the 1L media bottle, add:

- 15 g proteose peptone
- 1.5 g potassium phosphate dibasic ($K_2HPO_4$)
- 0.74 g magnesium sulfate ($MgSO_4$)
- 10 mL of 100% glycerol
- 15 g of agar (for solid media)

Add 890 mL of deionized water and mix thoroughly.

**Note:** Use ~200 mL of DI water in a 1L beaker to dissolve the base media components thoroughly. After transferring the solution to the 1L media bottle, rinse the beaker with ~100 mL of DI water to capture any remaining constituents. Repeat this rinsing process multiple times until all media components have been fully transferred, ensuring complete dissolution and accurate media composition.

2 **Prepare Boric Acid Solution**

In a 250 mL bottle, add:

- 1.5 g boric acid
- 100 mL of DI water

Place a stir bar in both bottles and mix at 500 rpm using a stir plate.

**Note:**

- Boric acid is difficult to dissolve in DI water, requiring prolonged stirring.
- If necessary, increase the mixing time or slightly warm the solution to aid solubility.
- Ensure the solution is fully mixed before proceeding to the next step.

3 **Sterilization**

Autoclave both bottles at 121°C for 15 minutes at 15 psi.

**Note:**

- Apply autoclave tape on the lid to confirm successful sterilization.
- Keep the lids slightly loose to allow pressure equilibration and prevent bottle explosion due to steam buildup during autoclaving.
- Ensure bottles are placed upright in the autoclave to prevent spills and contamination.

4 **Prepare Antibiotic Solutions**

1. While the media is autoclaving, prepare the antibiotics:

Cycloheximide solution (100 mg/mL):

- Dissolve 200 mg of cycloheximide in 2 mL of sterile DI water.

Cephalexin solution (10 mg/mL):

- Dissolve 80 mg of cephalexin in 8 mL of sterile DI water.

5 **Adding Antibiotics & Boric Acid Solution**

Allow the autoclaved medium to cool to ~50°C before proceeding.

Under a biosafety cabinet, add:

- 2 mL of cycloheximide solution
- 8 mL of cephalexin solution

**Note:** Rinse each antibiotic tube with autoclaved boric acid solution to ensure full transfer. Repeat twice before adding the remaining boric acid solution to the media.

6 **Pouring Plates**

- Under sterile conditions, pour 20–25 mL of medium per Petri dish.
- Allow plates to solidify at room temperature before sealing and storing.
- Store plates at 4°C in sealed plastic bags to prevent dehydration.

## Protocol references

KING, E. O., WARD, M. K., & RANEY, D. E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. *The Journal of laboratory and clinical medicine*, *44*(2), 301–307.