

M9 Medium with Kanamycin Preparation Protocol

Materials (per liter):

- **Na₂HPO₄·7H₂O**: 12.8 g
- **KH₂PO₄**: 3 g
- **NaCl**: 0.5 g
- **NH₄Cl**: 1 g
- **H₂O**: to 986 mL
- (If making plates, add 15 g of **agar**)

Add separately before use (per liter):

- **1M MgSO₄**: 2 mL
- **0.1 M CaCl₂**: 1 mL
- **50% glycerol**: 10 mL
- **1M IPTG**: 0.5 mL (optional, only if induction of expression is needed)
- **50 mg/mL kanamycin**: 1 mL

Instructions:

1. **Dissolve salts:**
 - In a 1 L beaker or flask, add 986 mL of deionized water.
 - Add the following chemicals in sequence, allowing each to dissolve fully before adding the next:
 - 12.8 g of **Na₂HPO₄·7H₂O**
 - 3 g of **KH₂PO₄**
 - 0.5 g of **NaCl**
 - 1 g of **NH₄Cl**
2. **Adjust the volume:**
 - If necessary, adjust the volume of the solution to 986 mL with deionized water.
3. **Autoclave:**
 - If making liquid M9 medium, autoclave the solution for 35 minutes at 121°C.
 - If making M9 agar plates, add 15 g of **agar** before autoclaving. Sterilize for 35 minutes at 121°C.
4. **Add supplements after cooling:**
 - Once the solution cools to ~55°C (to avoid denaturation of sensitive components), add the following sterile solutions:
 - **1M MgSO₄**: 2 mL
 - **0.1 M CaCl₂**: 1 mL
 - **50% glycerol**: 10 mL
 - **1M IPTG**: 0.5 mL (optional, only if induction of expression is needed)
 - **50 mg/mL kanamycin**: 1 mL
5. **Final volume:**
 - Adjust the final volume to 1 L with sterile deionized water if necessary.
6. **Storage:**
 - Store the prepared medium at 4°C if not using immediately. If plates were prepared, store them upside down at 4°C.

Your M9 medium with kanamycin is now ready to use for bacterial culture.