

Protocol for Preparing 50% Glycerol Stock Solution

Materials:

- Glycerol (100% or anhydrous glycerol)
 - Sterile deionized water (DI water)
 - 50 mL conical tube or sterile container
 - Pipettes and sterile tips
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Steps:

1. **Measure the glycerol:**
 - If working with **100% glycerol**, it is very viscous, so use a large pipette tip or pour it carefully. Measure **50 mL of glycerol**.
 2. **Add deionized water:**
 - Add **50 mL** of sterile deionized water to the glycerol to bring the final volume to **100 mL**. This will make a **50% glycerol solution**.
 3. **Mix thoroughly:**
 - Gently mix the glycerol and water until fully combined. This may take a bit of time due to the viscosity of the glycerol.
 4. **Sterilize (optional but recommended for some applications):**
 - If sterile conditions are required, filter the solution through a **0.22 µm sterile filter** to remove any potential contaminants.
 5. **Aliquot and store:**
 - Aliquot the solution into sterile 1.5 mL or 50 mL tubes as needed. Store the aliquots at **room temperature** or **4°C**.
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Note:

- 50% glycerol is commonly used as a cryoprotectant for bacterial and yeast stocks. For long-term storage, cultures are typically mixed with equal parts of the 50% glycerol stock (resulting in a final concentration of 15-20% glycerol) and stored at **-80°C**.