

Bacterial Growth Preparation

Caution: For Research Use. This product is intended for animal research only and not for use in humans. Not for human or animal therapeutic or diagnostic use.

IVISbrite™ Bacteria are shipped on agar plates not meant for long-term storage at -80 °C. Shortly after receipt, the bacteria should be prepared and stored as instructed below to ensure that the material does not become compromised.

Upon Receipt of the IVISbrite Bacteria Plate

1. Prepare a 15 mL tube capable of withstanding -80°C temperatures with fresh media/broth.
 - Prepare the broth as instructed by the vendor.
 - a. For *S. pneumoniae* use Brain Heart Infusion Broth (BHI)
 - b. For all other strains, use Luria Broth (LB)
2. Remove a single bacteria colony from the bacteria plate and inoculate the tube containing the broth. Repeat as desired based on the amount of material to be stored.
3. Grow overnight with shaking at 37 °C.
 - a. For *S. pneumoniae* incubate at 37 °C with 5% CO₂, shaking not necessary.
4. Media should be turbid the next day.
 - a. *S. pneumoniae* may grow slower, so an additional 24 hours of incubation is recommended.
5. Add 5 mL of sterile 30% glycerol to the tube and mix thoroughly. The final concentration of glycerol is now 15%.
6. Aliquot the material into multiple small vials. This allows for future use with minimal thawing.*
7. Store vials at -80 °C.

**Freeze-thaw cycles adversely affect the performance of the material, and should be avoided.*