Twist Glycerol Stocks Handling Instructions

Revival of frozen bacterial cultures requires careful handling due to their inherent sensitivity during the thawing process. Twist Bioscience glycerol stocks are manufactured in conjunction with the Clonal Gene delivered, utilizing proprietary cell lines (DH10B-like or Stbl3-like, depending on vector stability). To ensure successful growth, a gentle initial incubation period with a nutrient-rich medium is essential. It is highly recommended to streak, plate, and select single colonies from the glycerol stock to ensure cell recovery and minimize the chances of a mixed population in the final culture. Failure to adhere to these best practices may result in reduced performance. This document outlines the steps required to streak and inoculate glycerol stocks.

PROTOCOL COMPONENTS

Please read the product packaging and storage recommendations carefully for each kit and store components as recommended immediately upon arrival.

NAME	STORAGE
Glycerol stock	-80°C

MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to streak and inoculate glycerol stocks.

MATERIALS
REAGENTS AND CONSUMABLES
LB agar plate with appropriate antibiotic (see General Notes and Precautions section)
Liquid LB with appropriate antibiotic (see General Notes and Precautions section)
EQUIPMENT
P1000 pipette tips and compatible pipettes (or other material for streaking)
P2, P10, or P20 pipette tips and compatible pipettes (or other material for inoculation)
Static incubator
Shaking incubator

PROTOCOL OVERVIEW

This protocol begins with Twist glycerol stock and LB agar plates and generates starter cultures that can be used for subsequent experiments.

	TWIST BIOSCIENCE GLYCEROL STOCKS FOR PREPARING STARTER CULTURES	TIME
STEP 1	**Streaking Glycerol Stocks** Pickable colonies	**17 hours (including incubation)**
STEP 2	**Starter Culture Inoculation** Finalized starter cultures	**8.5 hours (including incubation)**

GENERAL NOTES AND PRECAUTIONS

Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol.

For best results, read this document before performing the protocol, and follow the instructions provided. Twist Bioscience cannot guarantee the performance of this workflow if modifications are made to the protocol.

Glycerol stocks should be kept cold at all times. When ready to use, transfer the stock directly from the -80°C freezer to ice to maintain stability.

- To refreeze, return the glycerol stock to the -80°C freezer as soon as possible after use. Minimize time at room temperature or on ice to preserve cell viability. Avoid repeated freeze-thaw cycles and aliquot stocks into smaller volumes if frequent use is expected.

Store light-sensitive antibiotic agar plates in foil to avoid loss of activity.

Recommended antibiotic concentrations for LB plates can be found in the table below:

ANTIBIOTIC	RECOMMENDED CONCENTRATION (mg/ml)	LIGHT SENSITIVE
Tetracycline	10	**YES**
Chloramphenicol	25	NO
Apramycin	50	NO
Kanamycin	50	NO
Zeocin	50	**YES**
Ampicillin	100	NO
Blasticidin	100	**YES**
Carbenicillin	100	NO
Spectinomycin	100	NO
Erythromycin	500	NO

STEP 1 STREAKING GLYCEROL STOCKS

Reagents Required

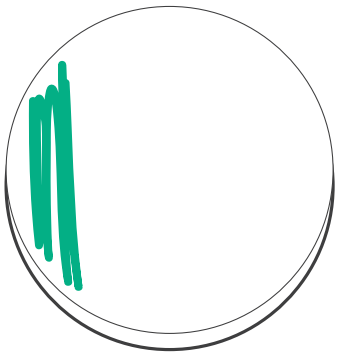
- LB agar plate with appropriate antibiotic
- Glycerol stock

Before You Begin

- Set a static incubator to 37°C (or other alternative growth temperature)
- Warm to 37°C (or other alternative growth temperature) for at least 30 minutes:
  - LB agar plate
- Place on ice:
  - Glycerol stock

1.1 Using a P1000 pipette tip (or other streaking tool of choice), scrape a small piece of the bacterial glycerol stock onto the streaking tool.

1.2 Using light pressure, spread the initial piece of the glycerol stock along the edge of the agar plate in a zigzag formation. Discard the pipette tip in an appropriate waste container.



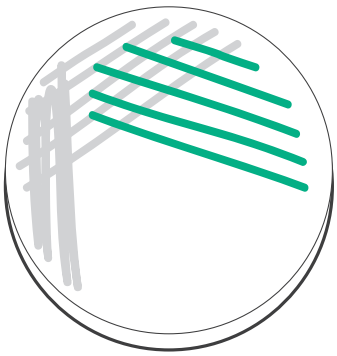
1.3 Using a new pipette tip, streak 5 separate lines from the initial streak. Discard the pipette tip in an appropriate waste container.

⚠IMPORTANT: Ensure light pressure is used. Do not pierce the surface of the agar.

1.4

Using a new pipette tip, streak 5 separate lines from the streak created in Step 1.3. Discard the pipette tip in an appropriate waste container.

⚠ IMPORTANT: Do not let the final streaks touch the initial streak location.

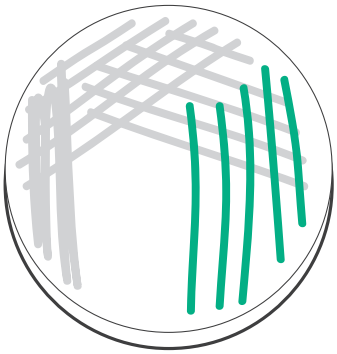


1.5

OPTIONAL: Skip to Step 1.7 if there is no space remaining on the plate

Using a new pipette tip, streak 5 separate lines from the streak created in Step 1.4. Discard the pipette tip in an appropriate waste container.

⚠ IMPORTANT: Do not let the final streaks touch the initial streak location.



1.6

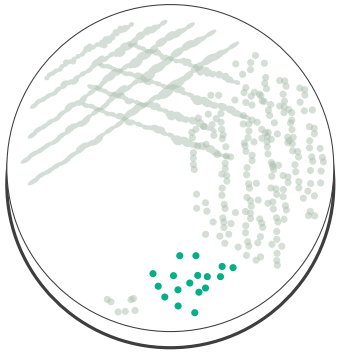
OPTIONAL: Skip to Step 1.7 if there is no space remaining on the plate

Using a new pipette tip, streak 5 separate lines from the streak created in Step 1.5. Discard the pipette tip in an appropriate waste container.

⚠ IMPORTANT: Do not let the final streaks touch the initial streak location.

1.7

If no alternative growth conditions apply, incubate at 37°C (or the recommended growth temperature) for a minimum of 16 hours. After incubation, inspect the colonies for growth. If the plate does not contain distinct colonies, Steps 1.1 to 1.7 will need to be repeated.
NOTE: The final streaked area should display well-isolated, relatively large colonies. Each colony is distinctly separate from the other by clear agar. These isolated colonies should also exhibit uniform macroscopic morphology, indicating a pure culture of a single species. If the colonies are small, too close together to pick individually, and/or appear to be two different sizes, Steps 1.1 to 1.7 will need to be repeated.



Note: This image is an idealized example. Actual results may differ depending on the bacterial species present.

STOPPING POINT: If not proceeding immediately to starter culture inoculation, store the agar plates with pickable colonies upside down at 4°C for up to a week. Keep the agar plate in foil if the antibiotic in use is light sensitive.

PROCEED TO STEP 2: STARTER CULTURE INOCULATION

STEP 2 STARTER CULTURE INOCULATION

Reagents Required

- Source plate with pickable colonies (from Step 1.7)
- 15 ml starter culture tube
- 2 ml of LB broth mixed with the appropriate antibiotic

Before You Begin

- Set a shaking incubator to 37°C and 300 rpm unless alternative growth conditions are needed
- Add 2 ml of LB broth mixed with the appropriate antibiotic to the 15 ml starter culture tube

2.1 Open the source plate and, with your free hand, use a P2, P10, or P20 pipette with a sterile tip (or other inoculation tool) to pick a well-isolated colony.

2.2 Open the starter culture tube and eject the tip (or other inoculation tool) into the tube and close the lid.

2.3 Plate the 15 ml culture tube into the 37°C incubator unless the vector requires special growth conditions.

2.4 If no other growth conditions apply, incubate at 37°C (or the recommended growth temperature) for a minimum of 8 hours. After incubation, inspect the culture for growth. If no visible and uniform evidence of growth is seen, Steps 2.1 to 2.4 will need to be repeated. If no pickable colonies remain on the source agar plate, then the whole protocol must be repeated.
NOTE: The starter culture should appear visibly and uniformly cloudy or turbid, indicating significant bacterial growth. Additionally, the culture should be pure and uniform. If there are visible clumps, layers, or multiple different colors present, Steps 2.1 to 2.4 will need to be repeated.

2.5 Once incubation is complete, the starter culture can be used as needed.

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