

# Vibrio Natriegens - Glycerol stock

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## Abstract

Preparation of *Vibrio natriegens* cell stocks for long-term storage at -80C. Principle is to remove any accumulated metabolic waste from overnight culture and resuspend in 20% glycerol final.

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## Before start

prepare 60% glycerol stock.

prepare LB3 media (high salt media composed of LB added with 3% NaCl final).

## Protocol

### Inoculate culture

#### Step 1.

Use a single colony to inoculate 1-3ml of LB3 media (add any required selection) and grow culture to density.

Culture can be grown at 30C or 37C shaker at 250rpm.

### Wash cells

#### Step 2.

Spin down 600uL of overnight culture at max speed for 1 min in room temp centrifuge.

Remove the supernatant leaving the cell pellet intact.

Resuspend the cell pellet with 400uL of growth media

Transfer to cryotube.

### Prepare glycerol stock

#### Step 3.

Add 200uL of 60% glycerol to cryotube, to get to 20% glycerol final.

Mix contents gently by rotating tube.  
Store tube at -80C