

Preparation of Liquid Hestrin Schramm (HS) Media

Introduction

This protocol creates the liquid growth medium used to grow *Komagataeibacter rhaeticus* iGEM. Following this protocol will yield 1L of HS media.

Reagents

- 1000 mL Milli-Q H₂O
- 20 g glucose
- 5 g yeast extract
- 5 g peptone
- 2.7 g disodium hydrogen phosphate
- 1.15 g citric acid

Equipment

- 1 L Erlenmeyer flask
- Analytical balance
- pH probe
- Aluminum foil
- Autoclave
- 0.22 µm syringe filter
- Syringe with threaded tip

Procedure

1. Measure out the indicated reagents.
2. Add 980 mL of Milli-Q H₂O to a 1 L Erlenmeyer flask.
3. Place yeast extract, peptone, disodium hydrogen phosphate, and citric acid into flask and swirl until components are thoroughly combined with water.
4. Measure the pH of the resulting solution. Adjust the solution to a pH of 6, titrating with NaOH if necessary.
5. Cover the mouth of the flask with aluminum foil, taking care not to contaminate the contents.
6. Autoclave the solution for 20 minutes. The next step can be done while waiting for the autoclave.
7. Mix the 20 g of glucose with 20 mL of Milli-Q H₂O to dissolve; you may need to place it on a hot plate to fully dissolve the glucose.
8. Filter the glucose/Milli-Q H₂O mix using a 0.22 µm filter tip attached to a syringe, adding the mix to the cooled down flask. Swirl to mix.
9. If not being used immediately, store in a 20% glycerol stock at -80°C.