

Spheroplasts

Introduction

This is a protocol for growing spheroplasts, cell wall-deficient *E.coli*, adapted from Chang *et al.*, 2018. Before making the overnight culture of *E.coli* that will be turned into spheroplasts, all of the components of the media that will be used in that step have to be prepared. Therefore, this protocol consists of several steps which are the following.

Steps

1. Sucrose solution
2. MSM media
3. Beef media
4. Cefsulodin stock solution
5. Overnight culture

Protocol

Sucrose solution 2 M (filter sterilized) 250 ml

Materials

- 250 ml water
- 171 g sucrose

Procedure

1. Add sucrose and water in a bottle.
2. Mix the ingredients with a magnet stirrer on a heating plate until the sugar is dissolved. Use low heat to avoid caramelization.
3. Filter-sterilize the solution with a Thermofisher Nalgene Vacuum filter. Connect the funnel to the vacuum tube in the fume hood. Measure the amount of solution in a measuring glass and add it to the funnel (do not put back the lid) and turn on the vacuum.

MSM media (500 ml)

Materials

- 250 ml water
- 1,9 g MgCl₂ (40 mM)
- 2,3 g Maleic acid (99%) (40mM)

- 250 ml of 2 M sucrose solution (1 M)

Procedure

1. Mix all the ingredients except sucrose in a sterilized bottle.
2. Check the pH for pH 7 otherwise regulate the pH by adding 10M NaOH.
3. Autoclave the bottle.
4. Add the 250 ml filter-sterilized sugar solution to the autoclaved bottle.
5. Store the bottle in a cold room if you are not going to use it right away.

Beef media (500 ml)

Materials

- 500 ml water
- 3g Beef extract
- 10g bacteriological peptone
- 5g yeast extract
- 5g NaCl

Procedure

1. Mix all the ingredients except for sucrose in a bottle.
2. Check pH for pH 7, otherwise regulate the pH.
3. Autoclave the bottle.
4. If not used right away, store it in a cold room.

Cefsulodin 0.045g/ml stock solution

Materials

- 0,045g cefsulodin
- 1 ml ddH₂O

Procedure

1. Add cefsulodin to the water in a 1.5 ml eppendorf tube and vortex.

Overnight culture

Materials

- 50 ml MSM medium
- 50 ml Beef medium
- 75 ul Cefsulodin
- 100 ul plasmid/bacteria specific antibiotics
- 1 ml overnight culture of *E.coli* (DH5α)

Procedure

1. Mix MSM and Beef media in an Erlenmeyer flask.
2. Add cefsulodin.
3. Add antibiotics that are specific to your *E.coli*. In general, 100 ul antibiotics should be added in 100 ml media (Beef + MSM).
4. Add 1 ml of *E.coli* overnight culture to the mixture.
5. Incubate the culture at 30°C for 20 hours.

References

Chang, H. J., Mayonove, P., Zavala, A., De Visch, A., Minard, P., Cohen-Gonsaud, M., & Bonnet, J. (2018). A modular receptor platform to expand the sensing repertoire of bacteria. *ACS synthetic biology*, 7(1), 166-175.