Are you sterile?

Standard Operating Procedure

Media Preparation for IVC Cultures

Laboratory: Biotechnology

Location: Ag Biotech Academy

SOP prepared by: R. Sanders

Last Revision: May 2024

General: The goal of this procedure is to prepare media containing antimicrobials and fungicides for controlling contamination in IVC cultures.

Safety: Safety Glasses

Materials

Murashige & Skoog Basal Media with sucrose & Gelzan (www.phytotechlab.com)
Fungicide (purchased at your local garden center)
Antibiotic-Antimycotic Solution (www.sigmaaldrich.com)
1 dram vials (Carolina Biological) containing the following media and labels:

Vial #1: Medium only
Vial #2: Medium with fungicide
Vial #3: Medium with antimicrobial agent
Vial #4: Medium with fungicide and antimicrobial agent

Procedures

To prepare Vial #1 medium:

1. Weigh out 1.8 g of Murashige & Skoog Basal Media.
2. Add the Murashige & Skoog Basal Media to 50 ml dH2O in a 150 ml Erlenmeyer flask.
3. Heat to boiling.
4. Allow to cool to approximately 600 C.
5. For Vial #1, pipette 2 ml into the vials labeled 1 using a sterile pipette.

To prepare Vial #2 medium:

6. Repeat steps 1-4.
7. Add 5 ml of the fungicide to the Murashige & Skoog Basal Media.
8. Pipette 2 ml into the vials labeled 2 using a sterile pipette.

To prepare Vial #3 medium:

9. Repeat steps 1-4.
10. Add 13 µl of the Antibiotic-Antimycotic Solution to the Murashige & Skoog Basal Media.
11. Pipette 2 ml into the vials labeled 3 using a sterile pipette.

To prepare Vial #4 medium:

12. Repeat steps 1-4.
13. Add 5 ml of the fungicide AND 13 µl of the Antibiotic-Antimycotic Solution to the Murashige & Skoog Basal Media.
14. Pipette 2 ml into the vials labeled 4 using a sterile pipette.