

## **PRACTICAL PROCEDURE FOR MAKING POTATO INOCULATION MEDIA**

### **A. TOOLS:**

1. Oven
2. Analytical scales
3. Hotplate and Magnetic Stirrer
4. Beaker glass 1000 ml
5. Measuring cylinder 100 ml
6. Measuring cylinder 10 ml
7. Drop pipette
8. Culture bottle
9. Autoclave
10. Gas stove
11. Clamp or gloves
12. Spoon

### **B. MATERIALS:**

1. Stock solution 1 (macro) , 2 (micro), 3 (Fe-EDTA) and 4 (Vitamin)
2. Sugar 40 gr/L
3. Agar 7 gr/L
4. Distilled water
5. NaOH
6. HCl
7. pH-Indicator
8. Heat resistant plastic
9. Label
10. Tissue
11. Rubber band

### **C. PREPARATION:**

1. Make media as much as 1 liter (1000 ml);
2. Prepare the tools and materials needed;

3. Ensure that the culture bottles have been sterilized in the oven for at least 1 hour with a temperature of at least 150°C;
4. Make sure the analytical scale is ready to use for weighing sugar and agar;
5. Make sure the hotplate and magnetic stirrer is ready to use to help dissolve the materials for making media;
6. Make sure you understand how to use a measuring pipette;
7. Make sure you understand how to use autoclave;
8. Perform making media according to the following procedures.

#### **D. PROCEDURES:**

1. Weigh 40 grams of sugar;
2. Weigh 7 grams of gelatine;
3. Prepare a 1000 beaker, insert the magnetic stirrer, place it on the hotplate;
4. Measuring for each stock solution, with the following conditions:
  - ❖ Stock solution 1 (macro) as much as 100 ml
  - ❖ Stock solution 2 (micro) as much as 10 ml
  - ❖ Stock solution 3 (Fe-EDTA) as much as 100 ml
  - ❖ Stock solution 4 (Vitamin) as much as 10 ml
5. Add distilled water up to 700 ml;
6. Put the sugar that has been weighed into the beaker;
7. Measure the pH of the media with pH indicator, if the pH of the media is less than 5,8 add NaOH, if the media pH is more than 5,8 add HCl (give drop by drop, use a pipette dropper). Make sure the pH of the media is 5,8;
8. Put agar that has been weighed into a beaker glass;
9. Adjust the media volume reaches 1000 ml in a beaker glass;
10. Press or turn the heating button on the hotplate, make sure that the media has dissolved completely;
11. Turn off the heating button after all the ingredients are completely dissolved and the media is boiling;
12. Remove the beaker from the hotplate;
13. Pour the media into a measuring cylinder as much as 10 ml, then put it in the culture bottle that has been prepared;

14. Close the culture bottle that already contains the media solution with heat resistant plastic, then tie it with a rubber band;
15. Sterilize the media in autoclave for 15 minutes (after the autoclave goes off);
16. Remove the media from the autoclave (before opening the autoclave cap, make sure the autoclave pressure has reached 0), then label the bottle by writing the type of media and date of making;
17. Store in the incubation room, after at least 1 week if no contamination, the media is ready to use;
18. Clean every tool that has been used;
19. Arrange the tools and materials that have been used orderly.

## **PRACTICE PROCEDURE OF MAKING POTATO SUBCULTURE MEDIA**

### **A. TOOLS:**

1. Oven
2. Analytical scales
3. Hotplate and Magnetic Stirrer
4. Beaker glass 1000 ml
5. Measuring cylinder 100 ml
6. Measuring cylinder 10 ml
7. Drop pipette
8. Culture bottle
9. Autoclave
10. Gas stove
11. Clamp or gloves
12. Spoon

### **B. MATERIALS:**

1. Stock solution 1 (macro) , 2 (micro), 3 (Fe-EDTA) and 4 (Vitamin)
2. Sugar 40 gr/L
3. Agar 7 gr/L
4. Distilled water
5. NaOH
6. HCl
7. pH-Indicator
8. Heat resistant plastic
9. Label
10. Tissue
11. Rubber band

### **C. PREPARATION:**

1. Make media as much as 1 liter (1000 ml);
2. Prepare the tools and materials needed;

3. Ensure that the culture bottles have been sterilized in the oven for at least 1 hour with a temperature of at least 150°C;
4. Make sure the analytical scale is ready to use for weighing sugar and agar;
5. Make sure the hotplate and magnetic stirrer is ready to use to help dissolve the materials for making media;
6. Make sure you understand how to use a measuring pipette;
7. Make sure you understand how to use autoclave;
8. Perform making media according to the following procedures.

#### **D. PROCEDURES:**

1. Weigh 40 grams of sugar;
2. Weigh 7 grams of gelatine;
3. Prepare a 1000 beaker, insert the magnetic stirrer, place it on the hotplate;
4. Measuring for each stock solution, with the following condition:
  - ❖ Stock solution 1 (macro) as much as 100 ml
  - ❖ Stock solution 2 (micro) as much as 10 ml
  - ❖ Stock solution 3 (Fe-EDTA) as much as 100 ml
  - ❖ Stock solution 4 (Vitamin) as much as 10 ml
5. Add distilled water up to 700 ml;
6. Put the sugar that has been weighed into the beaker;
7. Measure the pH of the media with pH indicator, if the pH of the media is less than 5,8 add NaOH, if the media pH is more than 5,8 add HCl (give drop by drop, use a pipette dropper). Make sure the pH of the media is 5,8;
8. Put agar that has been weighed into a beaker glass;
9. Adjust the media volume reaches 1000 ml in a beaker glass;
10. Press or turn the heating button on the hotplate, make sure that the media has dissolved completely;
11. Turn off the heating button after all the ingredients are completely dissolved and the media is boiling;
12. Remove the beaker from the hotplate;
13. Pour the media into a measuring cylinder as much as 20 ml, then put it in the culture bottle that has been prepared;

14. Close the culture bottle that already contains the media solution with heat resistant plastic, then tie it with a rubber band;
15. Sterilize the media in autoclave for 15 minutes (after the autoclave goes off);
16. Remove the media from the autoclave (before opening the autoclave cap, make sure the autoclave pressure has reached 0), then label the bottle by writing the type of media and date of making;
17. Store in the incubation room, after at least 1 week if no contamination, the media is ready to use;
18. Clean every tool that has been used.
19. Arrange the tools and materials that have been used orderly.