

## **PRACTICE PROCEDURE OF MAKING TOMATOES INOCULATION MEDIA**

### **A. Tools:**

1. Oven
2. Analytical scales
3. Hotplate and magnetic stirrer
4. Beaker glass 1000 ml or 2000 ml
5. Beaker glass
6. Measuring cylinder
7. Pipette 10 ml with dropper ball
8. Pipette 5 ml with dropper ball
9. Drop pipette
10. Culture bottle
11. Autoclave
12. Gas stove
13. Clamp or gloves
14. Container or aluminium bucket
15. Spoon

### **B. Materials:**

1. Stock solution 1 (macro) , 2 (micro), 3 (Fe-EDTA) and 4 (Vitamin)
2. Sugar 30 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

### **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 30 grams of sugar;
2. Weigh 7 grams of gelatine;
3. Prepare a 1000 ml or 2000 ml beaker, insert the magnetic stirrer, place it on the hotplate;
4. Enter approximately 500 ml of distilled water into a beaker;
5. Press or turn on a button hotplate at medium speed;
6. 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(Use a different pipette for each stock solution, or use the same pipette but before using it in another stock solution, rinse first with distilled water at least three times);
7. Put the sugar that has been weighed into the beaker;
8. 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;
9. Put agar that has been weighed into a beaker glass;
10. Adjust the media volume reaches 1000 ml in a beaker glass;
11. Press or turn the heating button on the hotplate, make sure that the media has dissolved completely;
12. Turn off the heating button after all the ingredients are completely dissolved and the media is boiling;
13. Remove the beaker from the hotplate;
14. Pour the media into a measuring cylinder as much as 20 ml, then put it in the culture bottle that has been prepared;
15. Close the culture bottle that already contains the media solution with heat resistant plastic, then tie it with a rubber band;

16. Sterilize the media in autoclave for 15 minutes (after the autoclave goes off);
17. 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;
18. Store in the incubation room, after at least 1 week if no contamination, the media is ready to use;
19. Clean every tool that has been used;
20. Arrange the tools and materials that have been used orderly.

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

### **A. Tools:**

1. Oven
2. Analytical scales
3. Hotplate and magnetic stirrer
4. Beaker glass 1000 ml or 2000 ml
5. Beaker glass 100 ml
6. Measuring cylinder 10 ml
7. Pipette 10 ml with dropper ball
8. Pipette 5 ml with dropper ball
9. Drop pipette
10. Culture bottle
11. Autoclave
12. Gas stove
13. Clamp or gloves
14. Container or aluminium bucket
15. Spoon
16. Plastic bucket

### **B. Materials:**

1. Stock solution 1 (macro) , 2 (micro), 3 (Fe-EDTA) and 4 (Vitamin)
2. Sugar 30 gr/L
3. Agar 7 gr/L
4. Growth regulator (BAP and IAA)
5. Distilled water
6. NaOH
7. HCl
8. pH indicator
9. Heat resistant plastic
10. Label
11. Tissue
12. 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 30 grams of sugar;
2. Weigh 7 grams of agar;
3. Prepare a 1000 ml or 2000 ml beaker, insert the magnetic stirrer, place it on the hotplate;
4. Enter approximately 500 ml of distilled water into a beaker;
5. Press or turn on a button hotplate at medium speed;
6. 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(Use a different pipette for each stock solution, or use the same pipette but before using it in another stock solution, rinse first with distilled water at least three times);
7. Measuring stock solution IAA, BAP, and IBA as a growth regulating;
8. Put the sugar that has been weighed into the beaker;
9. Measure the pH off 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;
10. Put agar that has been weighed into a beaker glass;
11. Adjust the media volume reaches 1000 ml in a beaker glass;
12. Press or turn the heating button on the hotplate, make sure that the media has dissolved completely;
13. Turn off the heating button after all the ingredients are completely dissolved and the media is boiling;
14. Remove the beaker from the hotplate;

15. Pour the media into a measuring cylinder as much as 20 ml, then put it in the culture bottle that has been prepared;
16. Close the culture bottle that already contains the media solution with heat resistant plastic, then tie it with a rubber band;
17. Sterilize the media in autoclave for 15 minutes (after the autoclave goes off);
18. 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;
19. Store in the incubation room, after at least 1 week if no contamination, the media is ready to use;
20. Clean every tool that has been used;
21. Arrange the tools and materials that have been used orderly.

## **PRACTICE PROCEDURE OF MAKING HOT PEPPERS INOCULATION MEDIA**

### **A. Tools:**

1. Oven
2. Analytical scales
3. Hotplate and magnetic stirrer
4. Beaker glass 1000 ml or 2000 ml
5. Beaker glass
6. Measuring cylinder
7. Pipette 10 ml with dropper ball
8. Pipette 5 ml with dropper ball
9. Drop pipette
10. Culture bottle
11. Autoclave
12. Gas stove
13. Clamp or gloves
14. Container or aluminium bucket
15. Spoon

### **B. Materials:**

1. Stock solution 1 (macro) , 2 (micro), 3 (Fe-EDTA) and 4 (Vitamin)
2. Sugar 30 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

### **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 30 grams of sugar;
2. Weigh 7 grams of gelatine;
3. Prepare a 1000 ml or 2000 ml beaker, insert the magnetic stirrer, place it on the hotplate;
4. Enter approximately 500 ml of distilled water into a beaker;
5. Press or turn on a button hotplate at medium speed;
6. 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(Use a different pipette for each stock solution, or use the same pipette but before using it in another stock solution, rinse first with distilled water at least three times);
7. Put the sugar that has been weighed into the beaker;
8. 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;
9. Put agar that has been weighed into a beaker glass;
10. Adjust the media volume reaches 1000 ml in a beaker glass;
11. Press or turn the heating button on the hotplate, make sure that the media has dissolved completely;
12. Turn off the heating button after all the ingredients are completely dissolved and the media is boiling;
13. Remove the beaker from the hotplate;
14. Pour the media into a measuring cylinder as much as 20 ml, then put it in the culture bottle that has been prepared;
15. Close the culture bottle that already contains the media solution with heat resistant plastic, then tie it with a rubber band;

16. Sterilize the media in autoclave for 15 minutes (after the autoclave goes off);
17. 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;
18. Store in the incubation room, after at least 1 week if no contamination, the media is ready to use;
19. Clean every tool that has been used;
20. Arrange the tools and materials that have been used orderly.

## **PRACTICE PROCEDURE OF MAKING HOT PEPPERS SUBCULTURE MEDIA**

### **A. Tools:**

1. Oven
2. Analytical scales
3. Hotplate and magnetic stirrer
4. Beaker glass 1000 ml or 2000 ml
5. Beaker glass 100 ml
6. Measuring cylinder 10 ml
7. Pipette 10 ml with dropper ball
8. Pipette 5 ml with dropper ball
9. Drop pipette
10. Culture bottle
11. Autoclave
12. Gas stove
13. Clamp or gloves
14. Container or aluminium bucket
15. Spoon
16. Plastic bucket

### **B. Materials:**

1. Stock solution 1 (macro) , 2 (micro), 3 (Fe-EDTA) and 4 (Vitamin)
2. Sugar 30 gr/L
3. Agar 7 gr/L
4. Growth regulator (BAP, NAA, TDZ)
5. Distilled water
6. NaOH
7. HCl
8. pH indicator
9. Heat resistant plastic
10. Label
11. Tissue
12. 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;
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8. Perform making media according to the following procedures.

**D. Procedures:**

1. Weigh 30 grams of sugar;
2. Weigh 7 grams of agar;
3. Prepare a 1000 ml or 2000 ml beaker, insert the magnetic stirrer, place it on the hotplate;
4. Enter approximately 500 ml of distilled water into a beaker;
5. Press or turn on a button hotplate at medium speed;
6. 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(Use a different pipette for each stock solution, or use the same pipette but before using it in another stock solution, rinse first with distilled water at least three times);
7. Measuring stock solution BAP, NAA, and TDZ as a growth regulating;
8. Put the sugar that has been weighed into the beaker;
9. 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;
10. Put agar that has been weighed into a beaker glass;
11. Adjust the media volume reaches 1000 ml in a beaker glass;
12. Press or turn the heating button on the hotplate, make sure that the media has dissolved completely;
13. Turn off the heating button after all the ingredients are completely dissolved and the media is boiling;
14. Remove the beaker from the hotplate;

15. Pour the media into a measuring cylinder as much as 20 ml, then put it in the culture bottle that has been prepared;
16. Close the culture bottle that already contains the media solution with heat resistant plastic, then tie it with a rubber band;
17. Sterilize the media in autoclave for 15 minutes (after the autoclave goes off);
18. 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;
19. Store in the incubation room, after at least 1 week if no contamination, the media is ready to use;
20. Clean every tool that has been used;
21. Arrange the tools and materials that have been used orderly.