

## **POTATO EXPLANT STERILIZATION**

### **A. TOOLS:**

1. Laminar Air Flow Cabinet (LAFC)
2. Beaker Glass
3. Tweezers
4. Stirrer
5. Jam bottles

### **B. MATERIALS:**

1. Explants (GO potato tubers that have sprouted)
2. Distilled water
3. Detergent or dishwashing liquid soap 1 tablespoon/liter
4. Clorox 10%
5. Twin 20 3 ml/100 ml
6. Bactericides 2 gr/liter
7. Fungicides 2 gr/liter
8. Alcohol 70%

### **C. PROCEDURES:**

1. Make sure all tools to be used have been sterilized;
2. Make sure the LAFC has been sterilized, as contained in the equipment sterilization procedure;
3. Prepare all the tools and materials needed;
4. Put the explant into the beaker glass;
5. Wash explants with running water (using plain water);
6. Soak the explants with detergent or dishwashing liquid, stir or shake for 5 minutes, then rinse 3 times with plain water until the foam is vanish;
7. Soak the explants with fungicide for 10 minute, then rinse 3 times with sterile distilled water (do this activity in LAFC);
8. Soak the explants with baktericide for 10 minute, then rinse 3 times with sterile distilled water (do this activity in LAFC) ;

9. Soak the explants with Twin 20 for 10 minute, then rinse 3 times with sterile distilled water (do this activity in LAFC);
10. Soak the explants with clorox 10% for 10 minute, then rinse 3 times with sterile distilled water (do this activity in LAFC);
11. Soak the explants with alcohol 70% for 10 minute, then rinse 3 times with sterile distilled water (do this activity in LAFC);
12. Transfer the explants to a new beaker glass or jam bottle, (do this activity in LAFC);
13. Remove all sterilization tools and materials from the LAFC except explants;
14. Clean and tidy up LAFC;
15. Spray LAFC again with 70% alcohol before inoculation the explants;
16. Explants are ready to be planted (next step refer to the practice procedure for planting potato explants).

## POTATO EXPLANTS INOCULATION

### A. TOOLS:

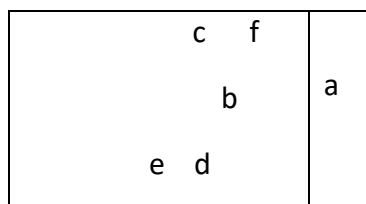
1. Laminar Air Flow Cabinet (L AFC)
2. Beaker glass 1000 ml or 2000 ml
3. Petridish
4. Tweezers
5. Scalpel + blade
6. Handsprayer
7. Bunsen burner
8. Jam bottle
9. Trolley rack

### B. MATERIALS:

1. Sterilized explants
2. Planting media
3. 70% alcohol
4. Spiritus/Methanol
5. Heat-resistant plastic
6. Rubber band
7. Cloth

### C. LANGKAH KERJA:

1. Before planting, L AFC must have been sterilized for at least 1 hour by turning on the UV lamp and blower;
2. Spray L AFC with 70% alcohol before adding all the planting materials and tools;
3. Spray materials and tools with 70% alcohol before adding them to L AFC;
4. Arrange the materials and tools properly and neatly (see picture),



**Keterangan:**

- a. Planting media
  - b. Explant
  - c. Planting tools (Scalpel+blade and Tweezers in a jam bottle contain with alcohol)
  - d. Petridish
  - e. Bunsen lamp
  - f. Beaker glass (rubbish bin)
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5. Light the Bunsen burner;
  6. Spray petridish with 70% alcohol and burn (sterilized it) over a Bunsen burner;
  7. Sterilized the scalpel blade and tweezer over the Bunsen Burner;
  8. Take the potato explant from the beaker glass using tweezer;
  9. Put it in the petridish;
  10. Take the potato tuber shoots carefully, by slicing the edges of the shoots and prying the shoots with a scalpel + blade;
  11. Peel the explants with a scalpel + blade and tweezers several layers if the buds are large, but if the buds are small and difficult to peel, then there is no need to peel them;
  12. Throw away of the peeling or dirt that is in the petridish into the prepared beaker;
  13. Let the explants that have been peeled off remain in the petridish, close the petridish again;
  14. Put back the scalpel + blade and tweezers into the glass filled with alcohol;
  15. Take the culture bottle containing the planting media, open the bottle cap over the Bunsen burner;
  16. Sterilized the tweezers over a Bunsen burner;
  17. Take explants from petridish and put them in the planting medium, each bottle of media contains 2 explants;
  18. Cover the bottle with heat resistant plastic that has been sterilized over a Bunsen burner;
  19. Tie back with rubber band;
  20. Give label with the name of the planter, date of planting, type of explant and type of media;

21. If the explant planting process is complete, arrange the bottles on the rack in the incubation room neatly (transfer to the incubation room using a trolley rack);
22. Get all tools and materials out of LAFC;
23. Clean and spray again with 70% alcohol;
24. Turn off LAFC.

## POTATO PLANTLET SUB CULTURE

### **A. TOOLS:**

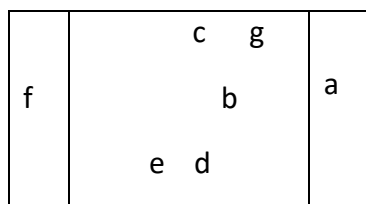
1. Laminar Air Flow Cabinet (L AFC)
2. Beaker glass 1000 ml or 2000 ml
3. Petridish
4. Tweezers
5. Scalpel + blade
6. Handsprayer
7. Bunsen burner
8. Jam bottle
9. Trolley rack

### **B. MATERIALS:**

1. Plantlet
2. Planting media
3. 70% alcohol
4. Spiritus/Methanol
5. Heat resistant plastic
6. Rubber band
7. Wiping cloth

### **C. PROCEDURES:**

1. Before planting, L AFC must have been sterilized for at least 1 hour by turning on the UV lamp and blower;
2. Spray L AFC with 70% alcohol before adding all the planting materials and tools;
3. Spray materials and tools with 70% alcohol before adding them to L AFC;
4. Arrange the materials and tools properly and neatly (see picture),



***Keterangan:***

- a. Planting media
- b. Plantlet
- c. Planting tools (Scalpel+blade and Tweezers in a jam bottle contain with alcohol)
- d. Petridish
- e. Burner bunsen
- f. Planting media that has been planted with explants
- g. Beaker glass (rubbish bin)

5. Light the Bunsen burner;
6. Spray petridish with 70% alcohol and burn (sterilized it) over a Bunsen burner;
7. Sterilized the scalpel blade and tweezers over the Bunsen Burner;
8. Take the potato plantlet from the bottle using tweezers;
9. Put it in the petridish;
10. Separate (cut) plantlets into parts according to shoot growth;
11. Arrange the cut plantlets neatly (pay attention to the top and bottom of the plantlets);
12. Discard the remaining pieces or roots that are in the petridish into the prepared beaker;
13. Let the plantlets that have been cut remain in the petridish, close the petridish;
14. Put back the scalpel + blade and tweezers into the glass filled with alcohol;
15. Take the planting medium, open the rubber and heat resistant plastic over the bunsen burner;
16. Burn the tweezers on the bunsen burner;
17. Take the plantlet pieces from the petridish and put them in the planting medium, each media bottle contains 5 plantlets;
18. Cover the bottle with preheated plastic;
19. Tie back with rubber;
20. Give label with the name of the planter, date of planting, type of explant and type of media;
21. When the subculture process has been completed, arrange the bottles on the rack in the incubation room neatly (use the trolley rack to carry the subculture results);
22. Get all tools and materials out of LAFC;

23. Clean and spray again with 70% alcohol;
24. Turn off LAFC.