Plant Cell
TECHNOLOGY

STARTER KIT

STEP-BY-STEP GUIDE

Starter Kit 2.0

Scan the QR Code for the Digital Guide & Video Tutorial:

1. What's Inside Your Starter Kit:

- **Supreme Agar (60 g)** – Solidifies your media. (5-9 g/L)
- **PPM™ (30 mL)** – Contamination control.
- **MS Media with Vitamins** – Makes 10 Liters of media. (1L = 4.43g)
- **10× Round snap-lock containers + lids** – Culture vessels.
- **5× Vented Glass Test tubes** – For small cultures or initiation stage.

- Plant Growth Regulators (PGRs):
  - **30 mL BAP** (cytokinin)
  - **30 mL IBA** (auxin)
  - **30 mL NAA** (auxin)

- Toolkit
  - **Scalpel + 1 blade**
  - **8" Forceps**
  - **4× Plastic pipettes**

2. What You'll Still Need to Gather:

- **Sterile Workspace** (*choose one*) - Still air box, Mini flow hood, **or** Laminar flow hood
- **Basic Tools & Supplies:**
  - Gram scale
  - Measuring spoons (⅛ tsp & ¼ tsp)
  - pH meter
  - 70% isopropyl alcohol
  - Face mask & gloves
  - Glass bead sterilizer **or** alcohol lamp + denatured alcohol *(not recommended for still air box)*
  - Pressure cooker **or** microwave
  - Table sugar (your regular sugar from the grocery store)
  - Bleach (your regular bleach from the grocery store)
  - 10mL Syringe

Optional:

- Extra test tubes/containers
- Extra scalpel blades
- Disposable blade remover
- Spray Bottle (for iso alcohol)

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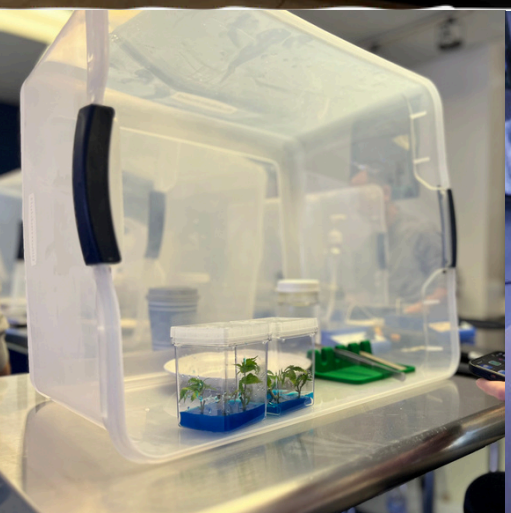
3. Setting Up Your Tissue Culture Area

You don't need to have all the fancy equipment to start tissue culture.

What you **do** need is a **clean, sterile environment** to protect your cultures from contamination.

In fact, **this was the first tissue culture "lab" our instructor Francisco built over 15 years ago**—and it was in a small, makeshift setup at home! He didn't have a professional laminar flow hood at the time, but he learned how to work clean and keep contamination low.



instagram.com/athena.ag

instagram.com/joes_exotic_plants

Your Options for a Sterile Workspace

- **Laminar Flow Hood** – Ideal for long-term, high-volume work (filters the air through a HEPA system).
- **Mini Flow Hood** – Smaller, more affordable, still offers filtered airflow.
- **Still Air Box (SAB)** – A DIY-friendly option that uses a clear box to block air currents so particles and contaminants can't reach your cultures.

DIY Still Air Box Ideas

If you're on a budget, you can make your own SAB at home.

Here's how some culturists have done it:

1. **Clear Plastic Storage Bin Method**
   - Choose a large, clear tote (upside down).
   - Cut two arm holes on one side and smooth the edges.
   - Work inside the bin with the lid on top, which keeps outside air from circulating.
2. **Acrylic Sheet Box**
   - Build a frame from PVC or wood.
   - Attach clear acrylic panels on all sides.
   - Cut arm holes and seal edges to prevent drafts.
3. **Glove Box Upgrade**
   - Similar to a SAB but with gloves permanently attached to the arm holes for extra protection. (Like the one Francisco built 15 years ago!)

SAB Use Tips for Best Results

- Work on a clean table in a **draft-free room** (no open windows, fans, or AC blowing).
- Wipe the inside walls, floor, and tools with **70% isopropyl alcohol** before each session.
- **Wear gloves and a mask** while working.
- **Avoid quick or sweeping arm movements inside**—move slowly to keep air still.

💡 **Pro Tip:** Many successful tissue culturists (including Francisco in his early days) started with nothing more than a still air box and the right technique.
The key is controlling airflow, working clean, and sterilizing your tools properly.

Additional Sterile Environment Ideas From Our Culturist Community

youtube.com/@Parkfolia
"Build A Simple Still Air Box Using Household Items! | Plant Tissue Culture at Home"
Watch at:
https://youtu.be/99pRX1vVXKU?si=QGh1Gh5iMA_mLT_T

instagram.com/plantsngreens
Using a still air box inside a greenhouse tent.
https://www.instagram.com/reel/C0hucOePf_L/?utm_source=ig_web_copy_link&igsh=MzRlODBiNWFlZA==

Additional Sterile Environment Ideas From Our Culturist Community

youtube.com/@PlantCellTechnology
“Plant Tissue Culturing From My Bathroom?!” by our Lab Director, Francisco youtube
Watch at:
https://youtu.be/1V4XjG5T78U

instagram.com/joes_exotic_plants
Using the mini flow hood to tissue culture rare plants in Puerto Rico

4. Aseptic Technique

Follow these practices to keep your workspace sterile and avoid contamination:

1. **No food or drinks** in the lab.
2. **Wear PPE:** gloves, mask (avoid long sleeves).
3. **Tie back long hair** and keep it covered.
4. **Work properly under the still air box or laminar flow hood:**
    - Sit straight, keep head out of the airflow.
    - Work at arm's length, as far back as possible.
    - Avoid broad arm movements over cultures.
    - Pass items carefully from one hand to another.
5. **Keep only necessary tools in the sterile area**—avoid clutter and blocking airflow.
6. **Disinfect** work surfaces before and after each session.
7. **Keep doors/windows closed** in the transfer area to prevent drafts.

5. Tips for Selecting Explants

- **Smaller explants** → Less contamination to remove.
- **Larger explants** → More tissue to help establish in culture.
- Use **new, actively growing shoots** whenever possible – they are cleaner than older woody stems.
- Plants grown **under cover** (greenhouse, indoors, or from forced dormant branches) are usually cleaner than field-grown plants.
- Explants from **younger plants** generally respond faster than those from older plants.
- Always use **sterilized tools** (dip in 10% bleach before use).
- For best results, **process explants immediately** after collection.

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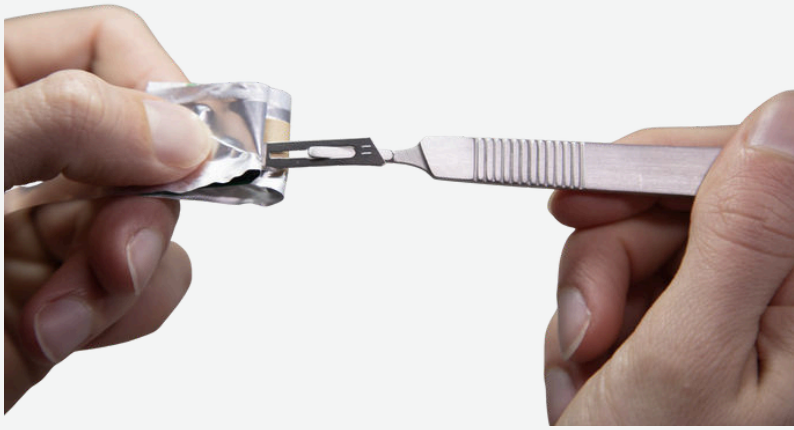
6. Explant Sterilization

Materials Needed:



- Scalpel and blade
- Bleach
- Distilled water
- 10 mL syringe
- Plants (explants)
- Medical gloves
- Medical mask

Steps:

- Cut plant sections with **at least one node.**
- Wash explants under running water with mild soap.
- Prepare a **9:1 bleach solution:**
  - 10 mL bleach + 90 mL water.
- Place explants in bleach solution for **10–15 min.** (You can use a round vessel from your starter kit for this step or any other container)
- **Rinse explants 3×** with sterile water.
- **Using your scalpel,** trim apical buds and remove as many leaf primordia as possible.
- Transfer explants to prepared culture media.

YOUR GUIDE TO:

PLANT GROWTH REGULATORS

AUXINS VS CYTOKININS

- High auxin-to-cytokinin ratio generally enhances **root formation.**
- **NAA** (Naphthaleneacetic Acid): Synthetic auxin, promotes root initiation.
- **IBA** (Indole-3-butyric acid): Synthetic, used for root initiation in difficult-to-root species.

- High cytokinin-to-auxin ratio generally enhances **shoot formation.**
- **BAP** (Benzylaminopurine): Synthetic cytokinin, used to promote shoot proliferation.

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7. Preparing Your Media

Instructions for 10x Round Snap-lock Vessels (*1 L of media total*):

- In a 1L container or beaker if you have one, **add 800 mL of distilled water.**
- To the water, **add 4.43 grams of PCT's MS Media with vitamins**, **30 grams of sugar** (*standard table sugar*), **1-2 mL/L of PPM™**, and the desired plant growth regulators.
- Add the rest of the water to **bring the total volume to 1 liter** and mix until all the ingredients have dissolved.
- **Adjust the pH** of the media **between 5.6 - 5.8** using KOH or another base (not included) to raise it and HCl or another acid (not included) to lower it.
- **Heat up the media close to boiling.**
- *Slowly* **add 6 grams of PCT's Supreme Tissue Culture Grade Agar** to the media and keep stirring until the Agar has dissolved.
- **Add 100 mL of media** to each vessel, mixing between pours, and ***loosely*** close the lids.
- **Autoclave the vessel** containing medium for **20 minutes at 15 psi** or 121°C.
- **Allow the media to cool and solidify** after autoclaving.

Your media is ready to start tissue culturing!

Instructions for 5x Vented Test Tubes (*100 mL of media total*):

- In one of the containers included in the kit, **add 80 mL of distilled water.**
- To the water, a**dd 0.443 grams of PCT's MS Media with vitamins**, **3 grams of sugar** (standard table sugar), **0.1-0.2 mL/L of PPM™**, and the desired plant growth regulators.
- Add the rest of the water to **bring the total volume to 100 mL** and mix until all the ingredients have dissolved.
- **Adjust the pH** of the media between **5.6 - 5.8.**
- **Heat up the media close to boiling.**
- *Slowly* **add 0.6 grams of PCT's Supreme Tissue Culture Grade Agar** to the media and keep stirring until the Agar has dissolved.
- **Add 25 mL of media** to each tube, mixing between pours, and ***loosely*** close the lids.
- **Autoclave the vessel** containing medium for **20 minutes at 15 psi** or 121°C.
- **Allow the media to cool and solidify** after autoclaving.

Your media is ready to start tissue culturing!

8. De-flasking & Acclimatization

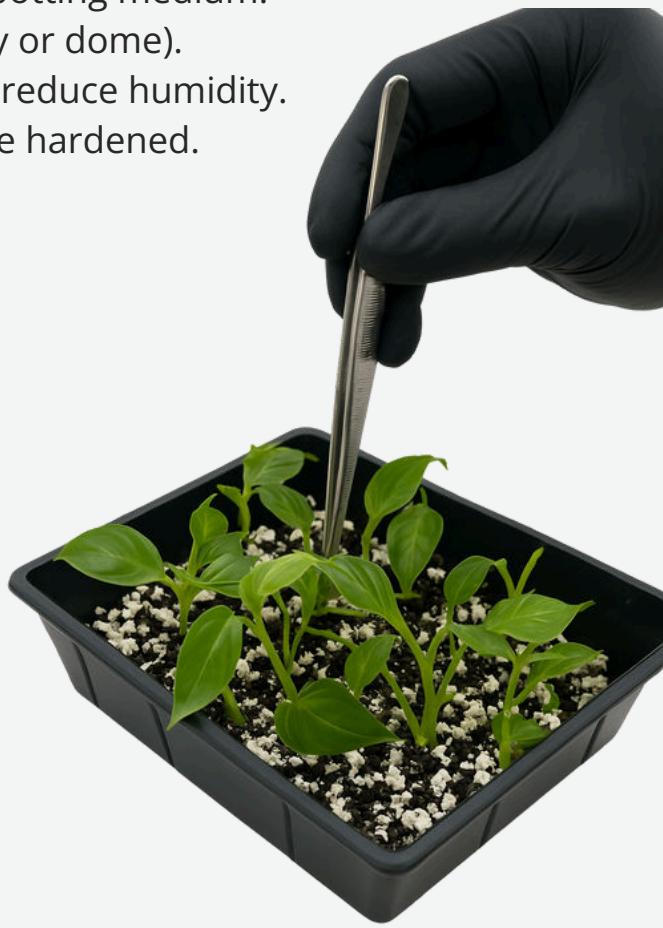
The goal of tissue culture is mass propagation—but most plant loss happens here if you're not careful.

Keys to Success:

- Keep **high humidity** at first, then gradually decrease it.
- *Slowly* **increase light intensity** over time.
- Remove all culture media from roots to avoid fungal growth.
- **Recommended medium:** Fluval Stratum with perlite mix (effective for many species).

Process:

1. Gently remove plantlets from culture vessels.
2. Rinse roots to remove all agar/media.
3. Plant into Fluval Stratum or your preferred potting medium.
4. Place under high humidity (e.g., covered tray or dome).
5. Over 1–2 weeks, open vents or lift covers to reduce humidity.
6. Increase light levels gradually until plants are hardened.



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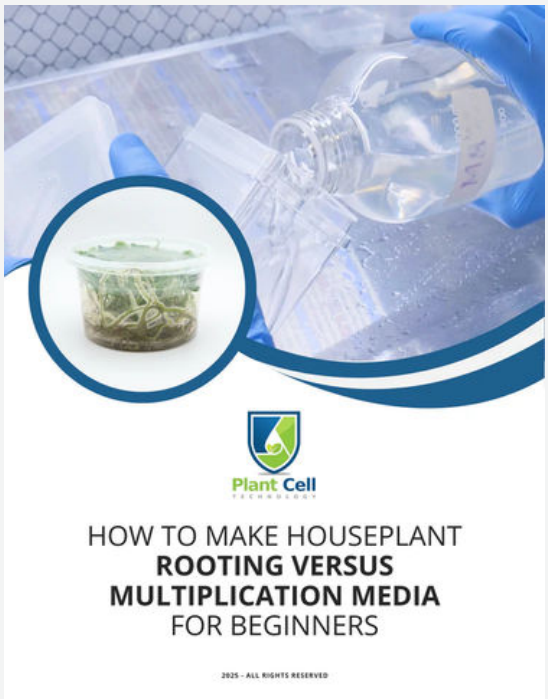
Additional Resources

How To Make Plant Tissue Culture Media: The Ultimate Guide (Beginner, Intermediate, and Pro): **https://youtu.be/0NyohDzVxqg?si=GrFbTku6Uhhu_wVV**



How To Make Houseplant Rooting Versus Multiplication Media For Beginners Guide

plantcelltechnology.com/blogs/free-resources/how-to-make-houseplant-rooting-versus-multiplication-media-for-beginners