

Plant propagation is the process of making new plants using existing plants. People are most familiar with propagation by dividing plants or putting leaves in water or soil, but another common form of propagation is tissue culture.

**Tissue culture or micropropagation**, is a method of making new plants "in vitro" on a nutrient media under sterile conditions. Leaves, stems, buds, seeds, roots, or even a few cells can develop into a full clone of the mother plant. Orchids are a common tissue culture plant.

Tissue culture is done under sterile conditions because fungi can grow on the nutrient media. This is often a barrier to doing tissue culture at home but sterilizing plant material, tools, and not breathing on the sterile medium can help.

#### **Supplies**

- Face mask
- Bleach
- Saucepan
- Rubbing alcohol

Homo Tissuo Culturo Modium

- Pressure-safe glass containers
- Dish soapLong-handled

Aluminum foil

• Glass jars with lids

- tweezers

  Razor blade
- Pressure cooker

In 1985, Mark P. Bridgen and Andrew Brand debuted a tissue culture media recipe for home tissue culture. With their new recipe, hobbyists could do tissue culture using ingredients more likely found at home such as table sugar and vitamin tablets.

# Make the Tissue Culture Medium

Wash hands and put on a face mask. Wash all of the glassware with a 10% bleach solution.

Combine all of the medium ingredients in a clean saucepan and boil 2 to 5 minutes until the agar dissolves. At this time, spray rubbing alcohol on the glassware and foil allowing it to evaporate.

Once the medium is finished, pour it into the glass containers and cover with foil. Also, prepare a glass jar with tap water and foil. Place all of the containers in a water bath in a pressure cooker at 15 psi for 15 minutes to sterilize.

Clean your work area with a 10% bleach solution and spray glass jars and lids with rubbing alcohol.

When finished, carefully release the pressure and use a hot pad to remove the containers. Pour the sterile medium into jars (Fig. 1, page 2), cap, and place in the refrigerator to set overnight or until use. Also refrigerate the sterile water until use.

nome rissue culture medium	
Medium Constituent	Amount (Mixture makes 1 pint of medium)
Table sugar Tap water All-purpose soluble fertilizer* Inositol tablet (250mg)** Vitamin tablet with thiamine Agar flakes	1/8 cup 1 cup 1 cup of stock solution ½ tablet, crushed ¼ tablet, crushed 2 tablespoons

\*Prepare the all-purpose soluble fertilizer stock solution by mixing ¼ tablespoons of a balanced, watersoluble fertilizer, such as 10-10-10, in a gallon of water. \*\*Inositol is sometimes sold as myo-inositol.

Table modified from Home Propagation Techniques, NDSU Extension. Source: Bridgen and Brand, 1985.



Fig. 1 Freshly poured media in sterilized jars prior to refrigeration. Photos by Bruce J. Black.

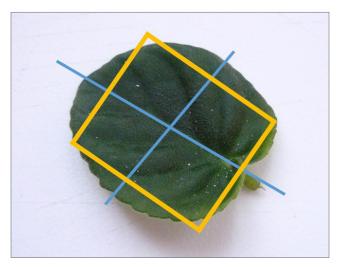


Fig. 2 African Violet (*Saintpaulia ionantha*) is an easy starter plant for micropropagation. Cut the leaves into smaller pieces after sterilization.

### **Plant selection**

Select what plant you would like to grow. African Violet (*Saintpaulia ionantha*) is an easy plant for beginners. Select healthy leaves, stems, or roots to propagate. Depending on the plant, one or multiple vegetative structures can be used to grow plants. For African Violet, leaves are most commonly used for at-home production.

### Prepare the plant material

Sterilize your work area and hands with rubbing alcohol. Then, sterilize all of the tools and plant material to prevent bacterial growth.

Wrap tweezers and razor blade in aluminum foil to make tool packets. This allows for easy removal and will keep the tools sterile. Sterilize the tool packets in boiling water or in the pressure cooker to remove pathogens. Let them cool while you sterilize the plant material.

Prepare a 10% bleach solution (using 450 ml of the sterile water and 50 ml of bleach) in a lidded container. Add 3 to 5 drops of dish soap. The soap prevents debris from sticking to the plant. Add the plant pieces and swirl the container for 15 minutes. Dump and refill with sterile water. Swirl to rinse. Repeat this process four times. Avoid breathing on the plant material or touching with unsterile hands.

Then, trim off the bleach-burnt edges with a razor blade and cut the plant material into smaller segments (Fig. 2). Discard the edge pieces.

## Add plants to the medium

Remove the medium from the refrigerator. Place the plant segments on the medium surface, pushing in slightly for leaf pieces and pushing in halfway for stem pieces. This will put more of the plant's surface area in contact with the media to take up nutrients.

Put the lid on, label jars with the date, and place in a 75°F room under light for 16 hours a day. If the room is cooler, plants will take longer to grow. If fungus forms and attacks plant material, discard contents.

Once the new plants outgrow the container, transfer them into a new container and begin acclimating them to their new environment ex-vitro.

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