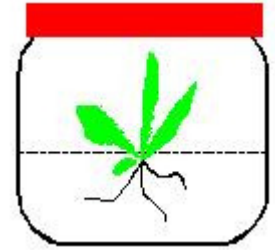


**Kitchen Culture Kits Inc.**  
Protocols for the Hobbyist  
[www.kitchenculturekit.com](http://www.kitchenculturekit.com)  
[carolstiff@kitchenculturekit.com](mailto:carolstiff@kitchenculturekit.com)



*“Bringing plant tissue culture to the classroom and home since 1998”*

**NOTE: All protocols are intended to be used following the methods described in the KCK Manual: “Plant Tissue Culture for the Classroom and Home” or the online workshop handout.**

## Hop (*Humulus lupulus* L.) Node Culture

Adapted from A.T.Roy, G. Leggett and A. Koutoulis. 2001. Development of a shoot multiplication system for Hop (*Humulus lupulus* L.). *In Vitro. Cell.Dev. Biol.-Plant* 37: 79-83.

### MEDIA

#### Initiation Medium:

In a 1 liter container, combine the following:

- \_\_\_\_\_ Distilled water - 2 cups or about 500 ml
- \_\_\_\_\_ MS Basal Medium with vitamins (1 liter packet)
- \_\_\_\_\_ PPM - 1 ml
- \_\_\_\_\_ Sucrose (table sugar) – 2 tablespoons
- \_\_\_\_\_ BAP – 0.5 ml (using solution of 1 mg/ml)
- \_\_\_\_\_ IAA – 0.1 ml (using solution of 1 mg/ml)

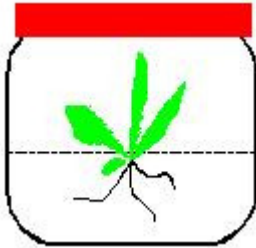
Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add 1 level “pinch” spoon of agar to each jar. **OPTIONAL: Dispense 2 tablespoons per baby food jar; add 1 level “smidgen” spoon of agar.** Sterilize via microwave or pressure cooker as described in the KCK manual.

#### Multiplication Medium (DO NOT MAKE UNTIL EXPLANTS ARE READY FOR NEW MEDIUM):

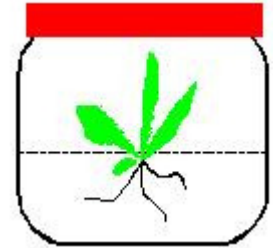
In a 1 liter jar, combine the following:

- \_\_\_\_\_ Distilled water - 2 cups or about 500 ml
- \_\_\_\_\_ MS Basal Medium with vitamins (1 liter packet)
- \_\_\_\_\_ PPM - 1 ml
- \_\_\_\_\_ Sucrose (table sugar) – 2 tablespoons
- \_\_\_\_\_ TDZ – 0.5 ml (using solution of 1 mg/ml)
- \_\_\_\_\_ IAA – 0.1 ml (using solution of 1 mg/ml)

Mix well, and then bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8.



**Kitchen Culture Kits Inc.**  
Protocols for the Hobbyist  
[www.kitchenculturekit.com](http://www.kitchenculturekit.com)  
[carolstiff@kitchenculturekit.com](mailto:carolstiff@kitchenculturekit.com)



*“Bringing plant tissue culture to the classroom and home since 1998”*

---

Dispense into baby food jars (3 tablespoons each). Add 1 level “pinch” spoon of agar to each jar. **OPTIONAL: Dispense 2 tablespoons per baby food jar; add 1 level “smidgen” spoon of agar.** Sterilize via microwave or pressure cooker as described in the KCK manual.

**Stage III Medium - Elongation**

(DO NOT MAKE UNTIL EXPLANTS ARE READY FOR NEW MEDIUM):

In a 1 liter jar, combine the following:

- \_\_\_\_\_ Distilled water - 2 cups or about 500 ml
- \_\_\_\_\_ MS Basal Medium with vitamins (1 liter packet)
- \_\_\_\_\_ PPM - 1 ml
- \_\_\_\_\_ Sucrose (table sugar) – 2 tablespoons

Mix well, bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add one level “pinch” of agar per jar. Sterilize via microwave or pressure cooker as described in the manual.

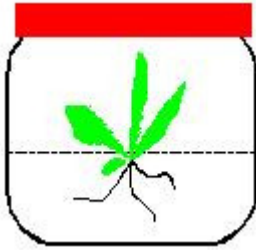
**Stage IV Medium – Rooting**

(DO NOT MAKE UNTIL EXPLANTS ARE READY FOR NEW MEDIUM):

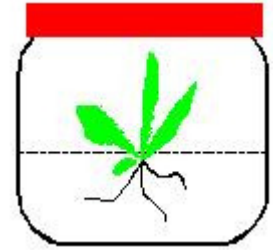
In a 1 liter jar, combine the following:

- \_\_\_\_\_ Distilled water - 2 cups or about 500 ml
- \_\_\_\_\_ ½ MS Basal Medium with vitamins ( use 1 tsp from 1 liter packet)
- \_\_\_\_\_ PPM - 1 ml
- \_\_\_\_\_ Sucrose (table sugar) – 2 tablespoons
- \_\_\_\_\_ IBA – 1 ml ((using solution of 1 mg/ml)
- \_\_\_\_\_ IAA – 1 ml ((using solution of 1 mg/ml)

Mix well, bring volume to 1 liter with distilled water. Adjust pH to 5.5 - 5.8. Dispense into baby food jars (3 tablespoons each). Add one level “pinch” of agar per jar. Sterilize via microwave or pressure cooker as described in the manual.



**Kitchen Culture Kits Inc.**  
Protocols for the Hobbyist  
[www.kitchenculturekit.com](http://www.kitchenculturekit.com)  
[carolstiff@kitchenculturekit.com](mailto:carolstiff@kitchenculturekit.com)



*“Bringing plant tissue culture to the classroom and home since 1998”*

---

**ISOLATION AND CULTURE OF EXPLANT:**

1. Collect young shoots of mature greenhouse grown plants.
2. Wash in running water for 30 minutes.
3. Section into node cuttings. Sterilize in 70% alcohol for 25-30 seconds.
4. Make bleach solution: 10 ml bleach + 90 ml water plus a few drops of detergent. Transfer node cuttings to bleach solution and stir for 10 minutes. *NOTE that the original paper recommended disinfecting with mercuric chloride which is hazardous and not recommended. NaDCC might also be tested here.*

**MOVE BLEACH SOLUTION/EXPLANT TO CLEANBOX**

5. Transfer explants from bleach solution to sterile water. Transfer to sterile water 2-3 more times (about 2 minutes each time).
6. Culture on initiation medium. Incubate at room temperature with 16 hours of light.
7. Explants are transferred to multiplication medium after 2-5 weeks.
8. When sufficient shoots have formed, these can be aseptically removed, placed on a sterile surface and sectioned into single shoots. These shoots are then elongated on Elongation Medium.
9. When shoots have sufficiently elongated a few inches, they can be transferred to Rooting Medium.
10. Successfully rooted plantlets transferred to sterile perlite:peat-moss:sand (1:1:1) and placed in greenhouse at 90-100% humidity for 3-4 weeks. Plants were then transferred into a shaded area for further acclimatization and grown to maturity.

See original publication for more details. **Note: KCK has not tested this protocol.**