

Media Preparation for *Artemisia tridentata* ssp. *tridentata*

Prepared by: Peggy Martinez

Adapted from: Dr. Marcelo Serpe

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The purpose of this protocol is to create a medium to grow surface sterilized seeds of *A. tridentata*. The volumes included are to make 200 mL of medium for each of 10 magenta boxes. Medium for each box will contain ½ MS+vitamin, 1% sucrose, 1 mL 1⁻¹ PPM and 0.3% phytigel with a pH of 5.7

Equipment:

Magenta boxes with lids (GA-7)

pH meter

Colored tape

Autoclave indicator tape

2L beaker or flask

Large graduated cylinder

Stir plate w/ stir bar

Dropper

Scale

Weigh boats

Spatula

1 mL pipette w/tips

Reagents:

Phytigel

Murashige & Skoog w/ Gamborg vitamins (MS)

Preservative for Plant tissue culture Media (PPM)

Sucrose

Potassium Hydroxide 0.1M

Step 1:

- Label magenta boxes using colored tape. For example:
- Mark each box with ~2 cm of autoclave indicator tape. Place this near the colored tape.

Step 2:

- Weigh 0.6g of phytigel into plastic weigh boat and add to each magenta box.

Step 3:

- Label 2L flask or beaker “MS media” and add 1L DI water using graduated cylinder. Place on stir plate with stir bar and turn on. No heat is needed.
- Weigh 4.4g MS+vitamin into weigh boat and add to flask.
- Weigh 20 g sucrose into weigh boat and add to flask.
- Add 2 mL PPM using pipette to solution in flask.

Step 4 (pH correction):

½ MS+vit + 1% sucrose + PPM + 0.3% phytigel pH 5.7
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- Add 1L (a tiny bit less than) of DI water to the media solution created in step 3.
- Place **calibrated** pH meter into solution (see separate document on how to calibrate pH meter).
- Add 0.1M KOH dropwise to flask until pH meter reads 5.7
 - NOTE: solution has little buffering capacity so wait for pH meter to stabilize before adding more 0.1M KOH. 5 drops at a time works well at this volume.
 - It is OK for pH to be a bit higher because autoclaving will slightly reduce pH value.
- After proper pH is reached remove pH meter, rinse with DI water and then return pH meter to buffering solution container.

Step 5:

- Pour 200 mL of media solution into each magenta box containing phytagel.
 - Pour media solution into graduated cylinder first for accuracy, a funnel can assist in this step.
- You may need to swirl the box slightly to mix phytagel with media solution.
- Place lids on boxes.
 - DO NOT tighten lids down completely. Place lids on securely, but do not press them on completely.
- Deliver to media prep room for autoclaving. This step takes an hour if they are able to autoclave them immediately.
- After autoclave, swirl media inside boxes slightly and press lids down tightly. Place media boxes back into lab in designated area.
 - NOTE: Boxes must not be opened unless under Laminar Flow Hood, especially since this solution contains sucrose (extra susceptible to contamination).

Reagents

Phytagel: CAS 71010-52-1; Sigma; powder

Murashige & Skoog w/ Gamborg vitamins: M404; Phytotechnology laboratories; phytolab.com; powder

Preservative for Plant tissue culture Media: aka PPM; plant cell technology; plantcelltechnology.com; liquid

Sucrose: Brand C&H sugar; granules

Potassium Hydroxide 0.1M: liquid