PhytoTech LABS

Product Information Sheet

P4001
PhytoAx™

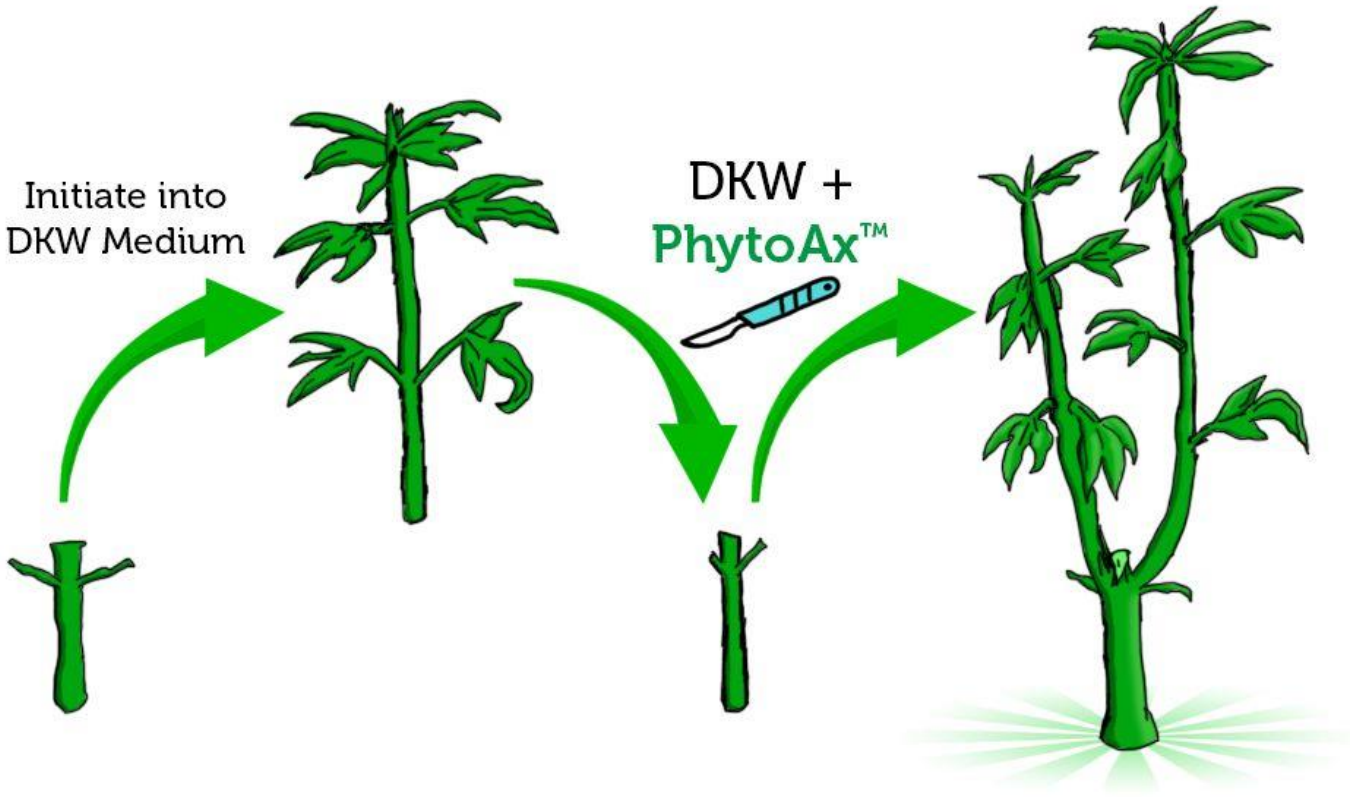
Properties:

Form:	Liquid
Appearance:	Clear, colorless
Application:	Plant Tissue Culture
Solubility:	Miscible with Water
Storage Temp:	-20°C
Typical Working Concentration:	0.5-1.0 mL/L, but the concentration should be determined by end user.

PhytoAx™ is a solution for tissue culture than can promote axillary shoots in cannabis or other species.

- High Fidelity Tissue-Replication
- Reproducible Multiplication-Rates
- Enhances Longevity in Culture

Patent Pending.



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Application Notes:

Directions for Seed-origin tissue:

1. Disinfect seeds. Please see Prod. No. E2620 Explant & Seed Decontamination Kit manual for more instructions.
2. Germinate seeds on the appropriate basal media (Appendix) until the seedling is at least 3-4 cm in height. Using 1/2X strength basal medium (e.g. 2.61 g/L D190) can improve germination efficiency.
3. Trim shoot-tips from germinated seedlings to be at least 1.0 cm in length, keeping the apical meristem.
4. Place shoot-tips on the appropriate basal media (Appendix) for 20-30 days in the growth room.
5. Prepare basal media (Appendix), adding 0.5-1.0 mL/L PhytoAx™ after the media has cooled to 45-50°C post autoclave.
6. Subculture growing stem tissue into nodes so that leaves are removed at petiole, the shoot apical meristem is removed, and tissue is at least 1.0 cm in length containing a leaf junction.
7. Place nodes on media for 20-30 days in the growth room.
8. Repeat steps 6-7 with media described in step 5 to maintain multiplication of plant tissue.

Directions for Vegetative (Clonal) tissue:

1. Trim branched shoots Clonal (Mother) plants to be at least 1.0 cm in length, and contain at least one node (i.e. stem containing the leaf junction approximately ½ to ¾ the way up the length of stem).
2. Remove the shoot apical meristem.
3. On each node, cut all the petioles halfway between the leaf and stem.
4. Disinfect nodes. Please see Prod. No. E2620 Explant & Seed Decontamination Kit manual for more instructions.
5. Place nodes on the appropriate basal media (Appendix) for 20-30 days in the growth room.
6. Subculture growing stem tissue into nodes so that leaves are removed at petiole, shoot apical meristem is removed, and tissue is at least 1.0 cm in length.
7. Prepare basal media (Appendix), adding 0.5-1.0 mL/L PhytoAx™ after the media has cooled to 45-50°C post autoclave.
8. Subculture growing stem tissue into nodes so that leaves are removed at petiole, the shoot apical meristem is removed, and tissue is at least 1.0 cm in length containing a leaf junction.
9. Place nodes on media for 20-30 days in the growth room.
10. Repeat steps 8-9 with media described in step 7 to maintain multiplication of plant tissue.

Please Note: While PhytoTech Labs Inc. tests each lot of this product with two or more plant cell/ tissue culture lines, it is the sole responsibility of the purchaser to determine the appropriateness of this product for the specific plants that are being cultured and applications that are being used.

Appendix:

Basal media – Mineral nutrient formulations such as DKW (often used with cannabis, Prod No. D2470, D190), Murashige & Skoog (used for the majority of herbaceous plant tissue, Prod. No. M519, M524), or WPM (woody plants, L449, L154) with only vitamins, carbohydrates, and a gelling agent.

An example basal media preparation would be as follows:

1. Measure out approximately 60% of the desired final volume of distilled/deionized water. For example: 600 ml for a final volume of 1000 ml. Add a magnetic stir bar to the vessel.
2. While stirring the water on a stir plate, add the powdered basal medium as indicated on the label (e.g 5.22 g/L of Prod. No. D190), and other non-heat labile components (1 mL/L of Prod. No. G219, 30 g/L of Prod. No. S391) and stir until completely dissolved.

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3. Add the gelling agent (e.g. 6-8 g/L of Prod. No. A111) slowly while stirring; it will not dissolve but should disperse into a uniform suspension. After all bubbles have liberated from the gelling agent, use distilled/deionized water to bring the medium to the final volume.
4. While stirring, determine the pH and adjust to 5.6-5.8.
5. Sterilize the medium in an autoclave at 121°C (15.1 psig), for approximately 30 min for 1L.
6. After removal from the autoclave, place the vessel on a magnetic stir plate and allow it to cool while stirring for 10-15 min.
7. Dispense the medium into sterile culture vessels under aseptic conditions after autoclaving. Tubes that are 25 x 150 mm would contain 20 mL, and larger vessels such as PTL-100's, or the like may contain approximately 75 mL. Steri-cons would contain approximately 125 mL.
8. Allow medium to gel for 3-5 hrs before adding any tissue to the medium.

PhytoAx™ medium preparation:

1. Measure out approximately 60% of the desired final volume of distilled/deionized water. For example: 600 ml for a final volume of 1000 ml. Add a magnetic stir bar to the vessel.
2. While stirring the water on a stir plate, add the powdered basal medium as indicated on the label (e.g 5.22 g/L of Prod. No. D190), and any other non-heat labile components (1 mL/L of Prod. No. G219, 30 g/L of Prod. No. S391) and stir until completely dissolved.
3. Add the gelling agent (e.g. 6-8 g/L of Prod. No. A111) slowly while stirring; it will not dissolve but should disperse into a uniform suspension. After all bubbles have liberated from the gelling agent, use distilled/deionized water to bring the medium to the final volume.
4. While stirring, determine the pH and adjust to 5.6-5.8.
5. Sterilize the medium in an autoclave at 121°C (15.1 psig), for approximately 30 min for 1L.
6. After removal from the autoclave, place the vessel on a magnetic stir plate and allow it to cool while stirring for 10-15 min.
7. Once cooled to approximately 45°C, add PhytoAx™ (0.5 – 1.0 mL/L, but the amount should be determined by the end-user) Continue stirring for 2-5 min.
8. Dispense the medium into sterile culture vessels under aseptic conditions after autoclaving. Tubes that are 25 x 150 mm would contain 20 mL, and larger vessels such as PTL-100's, or the like may contain approximately 75 mL. Steri-cons would contain approximately 125 mL.
9. Allow medium to gel for 3-5 hrs before adding any tissue to the medium.

Growth Room Environment:
Temperature: 25°C
Lighting: Fluorescent or LED ~40-50 $\mu mol/m^2s^1$ near the top leaf
Photoperiod: 16 hr on/8 hr off

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