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Version 2

Tomato Transformation Media V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol is simply the media recipes for use with my tomato transformation protocol.

Materials

MATERIALS

☒ Difco Bacto Agar **Carolina Catalog #156783B**

☒ Sucrose

☒ Luria broth powder

☒ Glycine **Bio Basic Inc. Catalog #GB0235.SIZE.500g**

☒ Nicotinic acid (NIACIN) **Bio Basic Inc. Catalog #NB0660.SIZE.250g**

☒ Myo-Inositol **Gold Biotechnology Catalog #I-525**

☒ Kinetin **Gold Biotechnology Catalog #K-100**

☒ Pyridoxine HCl (Vitamin B6) **Gold Biotechnology Catalog #P-780**

☒ Timentin™ Ticarcillin/Clavulanate (15/1) **Gold Biotechnology Catalog #T-104**

☒ Thiamine HCl **Gold Biotechnology Catalog #T-260**

☒ trans-Zeatin **Gold Biotechnology Catalog #Z-105**

☒ Murashige and Skoog with Nitsch Vitamins **Caisson Labs Catalog #MSP29-50LT**

☒ Murashige & Skoog Basal Salts **Caisson Labs Catalog #MSP01-50LT**

☒ Agargel **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A3301**

100mm x 15mm petri dishes (Fisher #FB0875713)

100mm x 20mm petri dishes (VWR #82050-918)

16oz soup containers with lids (Fabri-Kal **#9501034 and #9501070**)

Before start

It helps to make up stock solutions of the vitamins, hormones, and antibiotics ahead of time and freeze them.

- Since the concentration of phytohormones and vitamins can vary across medias, we make them both up to a standard concentration of 1mg/mL for easy math.
- Antibiotics are typically used at a single concentration in all of our medias, so they are made up as a 1000x stock. That is, their actual concentration varies depending on the antibiotic, but in every case you will add 1μL of stock for every 1mL of media.

- 1 To make 1L, for each media, dissolve the following components to ~800mL of MilliQ water in a 1L beaker

Component:	1/2MS O	MSO- 2%	KCM S	2Z	1Z	Rooti ng
MS Basal Salts	2.15g	4.3g	4.3g	--	--	--
MS Salts with Nitsch Vitamins	--	--	--	4.3g	4.3g	4.3g
Sucrose	10g	20g	30g	20g	20g	30g
myo-Inositol	100mg	100mg	100mg	100mg	100mg	--
KH ₂ PO ₄	--	--	200mg	--	--	--
Thiamine HCl (1mg/mL)	2mL	400μL	1.3mL	--	--	--
Pyridoxine HCl (1mg/mL)	500μL	500μL	--	--	--	--
Nicotinic Acid (1mg/mL)	500μL	500μL	--	--	--	--
Glycine	--	2mg	--	--	--	--
2,4-D (1mg/mL)	--	--	200μL	--	--	--
Kinetin (1mg/mL)	--	--	100μL	--	--	--

- 2 Bring volume to 1L and adjust pH (±0.03) according to the table below:

	1/2MS O	MSO- 2%	KCM S	2Z	1Z	Rootin g
pH	5.8	5.6	5.5	6.0	6.0	6.0

- 3 Divide the volume into two screw-top jars and add the following gelling agent. Note that not all medias require a gelling agent, and that this mass is the *TOTAL* mass added for 1L. It is not necessary to dissolve this powder; it will dissolve in the autoclave.

	1/2MS O	MSO- 2%	KCM S	2Z	1Z	Rooti ng
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Agar	8g	--	--	--	--	--
Agargel	--	--	5.2g	5.2g	5.2g	--
Difco Bacto Agar	--	--	--	--	--	8g

Only half this mass should go into each screw-top jar.

- 4 Autoclave the media, and allow to cool to ~50°. Add any antibiotics and phytohormones (filter-sterile) according to the table below:

	1/2MS O	MSO- 2%	KCM S	2Z	1Z	Rooti ng
Zeatin (1mg/mL)	--	--	--	2mL	1mL	--
Timentin (300mg/mL)	--	--	--	1mL	1mL	--
Antibiotics	No	No	No	Yes	Yes	Mayb e

- 5 In the sterile hood, dispense the media into the following containers. The liquid media can simply be stored in the screw-top jar.

	1/2MS O	MSO- 2%	KCM S	2Z	1Z	Rootin g
100×15mm Petri Dish	Yes	--	Yes	--	--	--
100×20mm Petri Dish	--	--	--	Yes	--	--
16oz Soup Container	--	--	--	--	Yes	Yes

- 6 Once the media has solidified, return the plates to their sleeves, label them accordingly, and store at 4°C for up to several months.