Tomato Transformation Media

Version 1

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dx.doi.org/10.17504/protocols.io.x6ffrbn

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ABSTRACT

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

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Difco Bacto Agar	156783B	Carolina
Sucrose		
Luria broth powder		
Glycine	GB0235.SIZE.500g	Bio Basic Inc.
Nicotinic acid (NIACIN)	NB0660.SIZE.250g	Bio Basic Inc.
Myo-Inositol	I-525	Gold Biotechnology
Kinetin	K-100	Gold Biotechnology
Pyridoxine HCl (Vitamin B6)	P-780	Gold Biotechnology
Timentin™ Ticarcillin/Clavulanate (15/1)	T-104	Gold Biotechnology
Thiamine HCl	T-260	Gold Biotechnology
trans-Zeatin	Z-105	Gold Biotechnology
Murashige and Skoog with Nitsch Vitamins	MSP29-50LT	Caisson Labs
Murashige & Skoog Basal Salts	MSP01-50LT	Caisson Labs
Agargel	A3301	Millipore Sigma

MATERIALS TEXT

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 STARTING

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:	1/2MSO	MS0-2%	KCMS	2Z	1Z	Rooting
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	--
KH2PO4	--	--	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:

	1/2MSO	MS0-2%	KCMS	2Z	1Z	Rooting
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.

	1/2MSO	MS0-2%	KCMS	2Z	1Z	Rooting
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:

	1/2MSO	MS0-2%	KCMS	2Z	1Z	Rooting
Zeatin (1mg/mL)	--	--	--	2mL	1mL	--
Timentin (300mg/mL)	--	--	--	1mL	1mL	--
Antibiotics	No	No	No	Yes	Yes	Maybe