




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Growing Arabidopsis on nutrient-rich media

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1 Works for me

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ABSTRACT

Notes on growing Arabidopsis seedlings on nutrient-rich media in plates.

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Prepare media

- 1 Select and prepare autoclaved media of choice (see recipes below). Make sure to pH before adding gelling agent.

A	B
MS basal salts	2.15 – 4.33 g/L
Sucrose	10 - 30 g/L
Vitamins (1000X) (optional)	1 mL/L
MES (optional)	0.1% (w/v)
KOH	pH 5.8
Agar/Phytoblend	8 g/L

MS Media (Murashige & Skoog, 1962)

A	B
Gamborg's B-5 basal medium	3.21 g/L
Sucrose	10 - 30 g/L
KOH	pH 5.8
Agar/Phytoblend	8 g/L

Gamborg B-5 Media (Gamborg et al 1968)

A	B
Kanamycin	30 - 50 uM
Basta (Glufosinate Ammonium)	20 - 50 uM

Supplement for transgenic selection - add to cooled autoclaved media immediately prior to pouring plates.

- 2 Pour room temperature media into plates in a biosafety cabinet - keep everything sterile! Allow media to cool and solidify, then package and store at 4 °C.


Surface sterilize seed

- 3 Collect small aliquots of desired Arabidopsis seeds into clean microfuge tubes.

- 4 

Add 500 uL of a solution made of 40% (v/v) household bleach, and 0.01% (v/v) Tween20 dissolved in distilled water and incubate for 5 minutes.

- 5 Remove supernatant, then rinse seeds with 700 uL of 70% ethanol incubating for 5-10 minutes. If you did not perform bleach sterilization step, repeat ethanol wash an additional 1-2 times.
- 6 Remove supernatant then rinse with distilled water at least 3 times.

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- 7 After final rinse, plate seeds out on media plates in sterile environment (e.g. BioSafety cabinet). Seeds can be stored at 4 °C for up to 1 month (maximum).