

Arabidopsis germination

MEDIUM

per liter

2.25 g MS salts (Sigma M5524) (in fridge)
10 g sucrose
pH 6.0 with KOH

for plates: add 2 g phytigel (final conc: 0.2%)
or: add 7 g agar (plant cell culture tested)

Antibiotics: 50 μ g/ml kanamycin
(to select for transformed plants, stock is 20 mg/ml in H₂O)

Surface sterilization of Arabidopsis seeds:

30 % bleach
0.1 % Triton X-100
for 5 to 10 min
rinse well at least 3 times with sterile H₂O

(can do direct stratification, or plate first)

Spreading of seeds:

Agarose method (0.1% in water):

Prepare sterile 0.1 % agarose (in water)

Add seeds to agarose (5 ml for large plates, 3 ml for small plates)

pour entire slurry onto plate, try to distribute seeds evenly

Single seed method:

Pick up sterile seeds in sterile 1000 μ l pipette tip

Let seeds settle to tip

Tap tip lightly on agar – this typically releases single seeds

If necessary, spread seeds with tip

After several seeds, need to set volume of pipetter down to release more seeds.

Stratification:

1 to 2 days in fridge.